THE NEED FOR MANGANESE IN THE BONE DEVELOPMENT OF THE CHICK

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ONE FIGURE

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Manganese was found by Wilgus, Norris and Heuser ('36, '37 a) to be markedly effective in preventing the incidence of perosis in chicks. The symptoms of perosis generally recognized are enlargement of the tibio-metatarsal joint, twisting and bending of the distal end of the tibia and of the proximal end of the tarso-metatarsus and slipping of the gastrocnemius tendon from its condyles.

In addition to these symptoms Herner and Robinson ('32) and Wilgus, Norris and Heuser ('37 b) reported that frequently the leg bones of chicks fed perosis-producing diets are thickened and shortened. In some instances the thickening and shortening of the leg bones was observed unaccompanied by other symptoms of perosis. On the other hand, Titus ('32) stated that in rickets the tarso-metatarsi and to some extent the tibiae are shortened and thickened whereas in perosis these bones are not affected in this manner. Also Holmes, Pigott and Moore ('33) measured the tibiae of normal chicks and of chicks afflicted with slipped tendon fed the same diet and concluded that the results obtained did not reveal any significant differences.

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Later Gallup and Norris ('38) made a preliminary report of the results of measuring the leg bones of chicks afflicted with perosis as a result of being fed a low-manganese diet and of normal chicks fed an adequate diet. They found that the chicks suffering from perosis had leg bones 7 to 8% shorter than those of the normal chicks of the same weight, sex and age. These workers also reported that a large percentage of the chicks hatched from eggs of low-manganese content have short leg bones. This observation is in agreement with a report by Lyons and Insko ('37) that chondrodystrophic embryos characterized by shortened and thickened legs, short wings and other deformities were produced from the eggs of hens fed a low-managanese diet. The difference between the length of the leg and wing bones of chondrodystrophic embryos and those of normal embryos was especially striking.

Although the results previously discussed are not in complete agreement it appeared probable that manganese plays a more important role in the bone development of the chick than merely the prevention of the anatomical deformities of the legs generally recognized as symptoms of perosis. Hence, a detailed study was initiated to determine the significance of the difference in bone development between chicks afflicted with perosis and normal chicks observed by Gallup and Norris ('38).

EXPERIMENTAL PROCEDURE AND RESULTS

Two lots of 100-day-old Rhode Island Red chicks were fed low-manganese (5.5 p.p.m.) and adequate-manganese (100 p.p.m.) diets respectively. The basal diet used was a modification of that used by Wilgus, Norris and Heuser ('37 b). The substitution of 20% of degerminated yellow cornmeal for a like amount of ground yellow corn and the replacement of fish meal with meat scrap were the principal changes in the diet.

At the end of 2 weeks and weekly thereafter for 4 weeks chicks approximately representative of the low-manganese lot

as determined by the weight distribution curve were paired with representative chicks of the same sex and weight from the adequate-manganese lot. The chicks were killed and the femurs dissected out with cartilage caps in place. Then the bones were measured, weighed individually and the amount of moisture, fat and ash determined.

The results presented in table 1 show that at 2 weeks of age the femurs of the Rhode Island Red chicks fed the low-manganese diet were approximately 1.8% shorter than those of the chicks of the same age, sex and approximately the same

TABLE 1

Effect of manganese on the development of the femurs of chicks[1]

MANGANESE IN DIET	CHICKS PER GROUP	AVERAGE WEIGHT	AVERAGE LENGTH OF FEMURS	DIFFERENCE	ODDS THAT DIFFERENCE IS SIGNIFICANT[2]
p.p.m.		*gm.*	*mm.*	%	
		Age 2 weeks			
5.5	11	120.0	33.8	1.8	17:1
100	11	118.6	34.4		
		Age 3 weeks			
5.5	11	172.5	38.0	7.5	713:1
100	11	173.0	41.1		
		Age 4 weeks			
5.5	9	222.8	42.3	8.7	3332:1
100	9	222.0	46.4		

[1] Rhode Island Red.

[2] Student's method.

weight fed the adequate-manganese diet. At 3 weeks of age the difference had increased to 7.5% and at 4 weeks to 8.7%. These results when analyzed by Student's method were found to be highly significant. Beyond 4 weeks of age no direct comparisons of bone length were made, since manganese deficiency was beginning to retard the growth rate seriously which would make it necessary to pair the more rapidly growing chicks of the low-manganese lot with the slower growing chicks of the adequate-manganese lot.

It was decided, therefore, to use chicks of different age but of the same weight and sex in making a series of com-

parisons. The results presented in table 2 show that, even though the low-manganese chicks were older than those with which they were compared, their femurs were 5.7% shorter, a difference which proved to be highly significant statistically.

In making the same type of comparison in another experiment with the exception that growth was retarded by a deficiency of riboflavin instead of manganese it was found that the older retarded chicks had the longer bones in spite of the fact that their weights were approximately the same as those of the younger, normal chicks. It appears, therefore, that the shortening of the bones in the low-manganese lot is not connected with the growth-retarding effect of a deficiency of manganese.

TABLE 2

Effect of manganese on the development of the femurs of chicks[1] *of the same weight but of different age*

MANGANESE IN DIET	CHICKS PER GROUP	AGE OF CHICKS	AVERAGE WEIGHT	AVERAGE LENGTH OF FEMURS	DIFFERENCE	ODDS THAT DIFFERENCE IS SIGNIFICANT[2]
p.p.m.		*wk.*	*gm.*	*mm.*	%	
5.5	15	7	529.4	54.4	5.7	311 : 1
100	15	6	520.5	57.7		

[1] Rhode Island Red.
[2] Student's method.

Since chicks fed a diet deficient in manganese are consistently retarded in growth, it was thought advisable to compare the bone length of two lots of retarded chicks, one of which was retarded in growth by a deficiency of manganese, the other by injection of excess quantities. The results of this study are presented in table 3. They show that the length of the femurs of the chicks, the growth of which was retarded by means of a low-manganese diet, was 8.3% shorter than that of the chicks whose growth was retarded by the injection of excess manganese. This difference by Student's method was found to be highly significant.

In the foregoing the comparisons of bone length were made between paired chicks. Since the chicks selected were not an

absolutely representative sample of the lot, it was felt desirable to use some method which would give a more complete comparison between the two lots as a whole. By plotting upon logarithmic paper the average length of the femurs against the average body weight of chicks from representative samples of the two lots curves were obtained the slopes of which gave the ratio of the increase in bone length to increase in body weight. The slope of the curve of the low-manganese lot was found to be less than that of the high-manganese lot. This showed that the retardation in growth of the bones in length is less than the retardation in increase in body weight of the chicks.

TABLE 3

Effect of manganese on the development of the femurs of retarded chicks [1]

RETARDED BY	CHICKS PER GROUP	AVERAGE WEIGHT	AVERAGE LENGTH OF FEMURS	DIFFERENCE	ODDS THAT DIFFERENCE IS SIGNIFICANT [2]
		gm.	*mm.*	%	
Low-manganese diet	7	365.0	44.3		
Injection of excess manganese	7	356.5	48.3	8.3	330: 1

[1] Rhode Island Red, age 6 weeks.

[2] Student's method.

The bones of the wings are affected by a deficiency of manganese the same as the bones of the legs but not quite to the same extent. At 3 weeks of age it was found that the average shortening of the femurs of chicks fed a low-manganese diet was 8.3%, the tibiae 9.5% and the tarso-metatarsi 5.7% whereas the average shortening of the humeri was 6.8%, the ulnae 6.7% and the metacarpi 2.2%. It was observed also that the spinal column of day-old chicks of hens fed a low-manganese diet is frequently shorter than that of day-old chicks of the same weight of hens fed an adequate diet. As a result of the effect of a deficiency of manganese upon bone development, the body conformation of the chick is altered, resulting in a stockier individual with a deeper body. In figure 1 are presented illustrations of the effect of

a deficiency of manganese upon the development of the bones of the legs and wings.

The results of twenty comparisons of the leg bones of White Leghorn chicks 4 weeks old agree essentially with those obtained in the studies on Rhode Island Red chicks. The tibiae of the White Leghorn chicks on the low-manganese diet averaged 2.6% shorter than those of their adequately fed

Fig. 1 Effect of manganese on the development of the leg and wing bones of chicks.

controls, and the tarso-metatarsi averaged 1.4% shorter. The smaller differences in bone length shown by this breed agree with the observation that White Leghorn chicks are less susceptible to perosis than the American breeds. Recent experiments showed that the manganese requirement of White Leghorn chicks is relatively low (Insko, Lyons and Martin, '38; Gallup and Norris, '39 a).

The essentialness of manganese for the normal development of bone in the chick is further emphasized by the results of measurements of the leg bones of day-old chicks hatched from the eggs of hens fed low-, medium- and high-manganese diets. The results are presented in table 4. They show that a manganese deficiency in the diet of hens results in the development of short leg bones in the chicks hatched from their eggs.

The chicks hatched from the eggs of two hens in the second low-manganese lot were exceptions, however, to this statement as they possessed leg bones of normal length. This is explained by the fact that upon analysis the eggs of these

TABLE 4

Effect of the manganese content of the maternal diet upon embryonic bone development

MANGANESE IN MATERNAL DIET	DAY-OLD CHICKS[1] PER GROUP	AVERAGE BONE LENGTH		DIFFERENCE	
		TIBIA	METATARSUS	TIBIA	METATARSUS
p.p.m.		*mm.*	*mm.*	%	%
13	16	26.0	18.7	12.5	14.2
53	6	29.7	21.8		
13	12	27.4	19.7	13.1	15.1
200	25	31.5	23.2		

[1] New Hampshire.

hens were found to contain 0.063 mg. of manganese per 100 gm. of dry egg yolk, presumably because of a more efficient transfer of manganese from the diet to the egg, while those of the other hens in this lot contained only 0.039 mg. of manganese per 100 gm. Gallup and Norris ('39 b) and others showed that hens fed a low-manganese diet generally lay eggs of low-manganese content.

The retardation in bone length in chicks fed a low-manganese diet cannot be explained on the basis of an increased water content in the body which would make it appear that relatively the body was developing faster than the bones. A study of the gross composition of chicks fed a low-manganese diet and of chicks fed an adequate-manganese diet at 4 weeks of age showed that the body composition of both

groups of chicks was approximately the same, the former containing 69.3% water, 5.81% fat and 3.90% ash and the latter containing 69.3% water, 4.91% fat and 3.93% ash.

In table 5 are presented the results of a study of the ash content of the femurs of representative chicks from the low-manganese and adequate-manganese lots. The ash content of the femurs of the low-manganese chicks decreased slightly with increase in age whereas that of the adequate-manganese chicks remained approximately constant. The differences in

TABLE 5

Effect of manganese on the ash content of the femurs of chicks[1]

MANGANESE IN DIET	CHICKS PER GROUP	AVERAGE WEIGHT	AVERAGE WEIGHT OF FEMURS	ASH IN FAT FREE DRY BONES	t VALUE[2]
p.p.m.		gm.	gm.	%	
		Age 2 weeks			
5.5	15	120.2	0.727	40.66	2.149
100	15	122.1	0.742	42.01	
		Age 3 weeks			
5.5	15	166.6	1.30	42.80	0.987
100	15	179.7	1.37	43.42	
		Age 5 weeks			
5.5	15	268.0	2.10	41.65	4.984
100	15	381.2	2.85	44.78	
		Age 6 weeks			
5.5	15	300.7	2.74	40.45	6.259
100	15	448.0	3.77	44.35	

[1] Rhode Island Red.

[2] For odds of 19 to 1 t must have a value of 1.990. For odds of 99 to 1 t must have a value of 2.638.

the ash content at 5 and 6 weeks of age are highly significant statistically. They are not due to difference in size of chick, since the bone-ash content of the low-manganese chicks at 6 weeks of age was less than that of the adequate-manganese chicks at 3 weeks of age at which time the former chicks weighed more than the latter. The results obtained in this study on the bone-ash content of chicks fed a low-manganese diet do not agree with those of other workers (Hogan, Shrewsbury and Kempster, '28; Hunter and Funk, '30; Hall and King, '31 and others) whose studies on the ash content of the

bones of chicks afflicted with perosis failed to reveal any marked variation from the normal.

The reduction in bone ash in the low-manganese lot was not due to rickets in the generally accepted meaning of the term. The diet fed the chicks in the low-manganese lot as well as that fed the adequate-manganese lot contained approximately 1.85% of calcium and 0.85% of phosphorus, amounts according to Wilgus ('31) in excess of the minimum requirements for normal bone formation. These diets also contained approximately 65 A.O.A.C. units of vitamin D per 100 gm., three times the minimum amount found necessary to prevent rickets by Murphy, Hunter and Knandel ('36). The serum calcium [3] of the chicks fed these diets was within the normal range of 10 to 12 mg. per 100 cc. of blood serum. In studies on the relation of vitamin D to the manganese requirement [4] it was observed that in chicks fed the same low-manganese diet plus 20 A.O.A.C. units of vitamin D per 100 gm. the calcification of the bones was normal upon examination in accordance with the procedure of McCollum, Simmonds, Shipley and Park ('22). Also no difference in calcification was observed by Gallup and Norris ('38) as a result of x-ray examination of the leg bones of chicks fed a low-manganese diet and those of chicks fed an adequate diet. For these reasons it appears highly improbable that manganese favorably influences vitamin D utilization. Rather it has been found by Caskey and Norris ('38) that the effectiveness of manganese in preventing perosis in chicks fed a high-calcium, high-phosphorus diet is decreased when an adequate amount of vitamin D is supplied.

In view of these considerations the conclusion that manganese along with vitamin D is concerned with calcium and phosphorus metabolism in the chick appears inescapable. This conclusion is supported by the results of Caskey and Norris ('38) showing that the breaking strength of eggs and egg-shell ash (CaO) are directly correlated with the manganese content of the diet fed the hens. It is also supported by

[3, 4] Unpublished results.

the results of Wiese, Johnson, Elvehjem and Hart ('38) who found that blood and bone phosphatase and ester phosphorus of the blood are depressed in manganese deficiency in the chick. The corollary of this conclusion is that the abnormal shortening of the bones of chicks is probably the direct effect of the upset in calcium and phosphorus metabolism resulting from manganese deficiency.

CONCLUSIONS

A deficiency of manganese in the diet of chicks results in a significant shortening of the bones of the legs and wings as well as a shortening of the spinal column. The shortening of the bones cannot be explained by an alteration in the gross composition of the body, since the body composition of both lots of chicks was found to be approximately the same.

It was also found that the ash content of the bones of chicks fed a low-manganese diet was significantly lower than that of chicks fed an adequate diet. This was not due to the development of a rachitic condition from defective vitamin D utilization, as the low-manganese diet contained excess vitamin D, serum calcium was within the normal range and examination of the leg bones by means of the x-ray and by staining sections with silver nitrate showed calcification to be normal.

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LITERATURE CITED

CASKEY, C. D., AND L. C. NORRIS 1938 Further studies on the role of manganese in poultry nutrition. Poul. Sci., vol. 17, p. 433.

GALLUP, W. D., AND L. C. NORRIS 1938 The essentialness of manganese for the normal development of bone. Science, vol. 87, p. 18.

———— 1939 a The amount of manganese required to prevent perosis in the chick. Poul. Sci., vol. 18, p. 76.

———— 1939 b The effect of a deficiency of manganese in the diet of the hen. Poul. Sci., vol. 18, p. 83.

HALL, G. E., AND E. J. KING 1931 Calcium and phosphorus metabolism in the chicken. Poul. Sci., vol. 10, p. 259.

HERNER, M. C., AND A. D. ROBINSON 1932 A study of leg-bone deformities in growing chicks. Poul. Sci., vol. 11, p. 283.

HOGAN, A. G., C. L. SHREWSBURY AND H. L. KEMPSTER 1928 Rapid growth of chicks on rations of natural foodstuffs. J. Agr. Res., vol. 37, p. 115.

HOLMES, A. D., MADLEINE G. PIGOTT AND W. B. MOORE 1933 Mineral content of tibiae from chicks with slipped tendon. Poul. Sci., vol. 12, p. 356.

HUNTER, J. E., AND E. M. FUNK 1931 The production of 'slipped tendon' in chicks by experimental feeding. Proc. Poul. Sci. Assoc., 22nd ann. meet., p. 45.

INSKO, W. M., JR., M. LYONS AND J. H. MARTIN 1938 The quantitative requirement of the growing chick for manganese. J. Nutrition, vol. 15, p. 621.

LYONS, M., AND W. M. INSKO, JR. 1937 Chondrodystrophy in the chick embryo produced by manganese deficiency in the diet of the hen. Ky. Agr. Exp. Sta. Bull. 371.

MCCOLLUM, E. V., N. SIMMONDS, P. G. SHIPLEY AND E. A. PARK 1922 A delicate biological test for calcium-depositing substances. J. Biol. Chem., vol. 51, p. 41.

MURPHY, R. R., J. E. HUNTER AND H. C. KNANDEL 1936 The vitamin D requirements of growing chicks and laying hens. Penn. Agr. Exp. Sta. Bull. 334.

TITUS, H. W. 1932 Perosis, or deforming leg weakness in the chicken. Poul. Sci., vol. 11, p. 117.

WILGUS, H. S., JR. 1931 The quantitative requirement of the growing chick for calcium and phosphorus. Poul. Sci., vol. 10, p. 107.

WILGUS, H. S., JR., L. C. NORRIS AND G. F. HEUSER 1936 The role of certain inorganic elements in the cause and prevention of perosis. Science, vol. 84, p. 252.

———— 1937 a The role of manganese and certain other trace elements in the prevention of perosis. J. Nutrition, vol. 14, p. 155.

———— 1937 b The effect of various calcium and phosphorus salts on the severity of perosis. Poul. Sci., vol. 16, p. 232.

WIESE, A. C., B. C. JOHNSON, C. A. ELVEHJEM AND E. B. HART 1938 Phosphorus metabolism of chicks afflicted with perosis. Science, vol. 88, p. 383.

CITRIC ACID AND ITS SALTS AS CALCIFYING AGENTS IN RATS

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The recent reviews of Schmidt and Greenberg ('35) and Gubner ('37) have summarized available information concerning calcium and phosphorus metabolism and their relations to rickets. They serve also to bring out the complexity of the problem of bone formation and to point out the conflicting views on how it is brought about.

Reports of early work on the effect of the acid-base value of the diet on rickets have been summarized by Morris, Ford and Graham ('36). Although their own experiments showed no evidence of acidosis as a causal or associated factor in infantile rickets, the earlier studies had shown conflicting results.

Early studies on the use of citric acid milk in preventing or curing infantile rickets also showed conflicting results. Weissenberg ('28) stated that his 'Zitronensäurevollmilch' prevented or cured rickets in the majority of cases, but Hess and Matzner ('24) did not find lemon juice milk effective without the addition of egg yolk.

The importance of citrates in calcification has received rather minor attention until the recent work of Shohl ('37) and Hamilton and Dewar ('37). Shohl showed by histological and x-ray studies that the addition of citric acid-sodium citrate or tartaric acid-sodium tartrate mixtures to rachito-

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genic diets will render them non-rachitogenic, and Hamilton and Dewar showed by the measurement of the width of the epiphyseal space on x-ray plates that the addition of sodium citrate, citric acid, sodium tartrate, sodium bitartrate or tartaric acid would give normal width to the epiphyseal line. They also secured normal or nearly normal line tests (3.5 or better) on rats fed these substances according to the vitamin D test technic (U. S. Pharmacopeia XI). These two papers differ in that the latter authors found the acids or the salts effective whereas Shohl found only the mixtures effective in producing normal x-ray and histological results.

Thus we see that the literature contains many contradictions, and in the light of these contradictions the present study of citric acid and citrates as calcifying agents was undertaken.

PLAN OF THE EXPERIMENT

Young albino rats from our own colony weighing 35 to 50 gm. at 21 to 25 days of age were placed in individual cages in a dark room. The animals in both experiments I and II were placed on the Steenbock-Black rachitogenic diet 2965 ('25) for 22 days. They were then given an experimental diet for 10 days and were killed on the eleventh day. The hind legs were dissected from the body, the femurs preserved in 95% ethyl alcohol, the tibias in acetone, for analysis for bone ash and line test respectively. Record of food intakes and body weights were made two to three times weekly throughout the study but no attempt was made to control food intakes. The experimental diets were prepared by adding to 100 gm. rachitogenic diet the following supplements:

Experiment I	mol.
1. None (controls)	
2. Citric acid	0.04
3. Sodium citrate	0.04
4. Citric acid + sodium citrate	0.02 0.02
5. Citric acid + sodium citrate	0.03 0.02
6. Lactic acid	0.04

Experiment II	mol.
1. None (controls)	
2. Potassium citrate	0.04
3. Citric acid + potassium citrate	0.02 0.02
4. Citric acid + potassium citrate	0.03 0.02

The technic used in the preparation and reading of the line tests was that of McCollum, Simmonds, Shipley and Park ('22). For the bone ash determinations each pair of femurs and the rat numbers were tied in small pieces of cheese cloth and extracted with ethyl alcohol for 48 hours in a soxlet extraction apparatus. They were then extracted with ether for 24 hours. The details of the method used for weighing and ashing the bones are given by Lachat ('35). The 1 hour heating was used, and the samples were reheated and reweighed as a check for complete ashing.

EXPERIMENTAL RESULTS

The results of these studies are given in table 1. They were separated into experiments I and II because the rats were from different litters, the initial weights of the animals were different and the food intakes of the animals in experiment II were greater both during the depletion and the experimental periods. It is of interest to note, however, that the average gains in weight of the corresponding groups are strikingly similar in spite of these differences.

Although the animals do not show complete agreement between line tests and bone ash values, the following summary may be made from the table of the probable effectiveness as antirachitic agents of the supplements used.

Since calcification in the controls was very poor, evidenced both by line test and bone ash values, the basal diet was truly rachitogenic. The addition of citric acid (0.04 mol. per 100 gm. basal ration) resulted in only limited improvement, more marked in the epiphyseal region than throughout the bone. The odds that the differences in bone ash values found are not due to chance are only 28:1, so that these results are barely significant. Sodium citrate (0.04 mol. per 100 gm. basal ration) and a mixture of equal parts of citric acid and sodium citrate (i.e., 0.02 mol. of each) averaged practically identical results and showed definitely better calcification than the citric acid alone, both by line test and by bone ash values. The odds for the significance of these bone ash values are

TABLE 1

The effect of acid and of acid-salt mixtures on calcification

SUPPLEMENT	LITTER	SEX	WEIGHT AT START OF EXPERIMENT	DEPLETION PERIOD 22 DAYS		EXPERIMENT PERIOD 10 DAYS		LINE TEST	BONE ASH
				Gain in weight	Food intake	Gain in weight	Food intake		
Experiment I									
			gm.	*gm.*	*gm.*	*gm.*	*gm.*		%
1. None (controls)	1	♀	34	18	121	12	67	R	22.0
	2	♂	46	30	144	18	82	R-1	28.6
	3	♂	40	24	121	6	63	R	23.2
	4	♀	38	14	97	11	54	R	26.3
	4	♀	40	16	110	4	50	R	24.0
		Av.	40	20	119	10	63	R	24.8
2. Citric acid	1	♂	38	14	98	10	56	2	29.6
0.04 mol. in	2	♀	43	23	122	8	68	2.5	30.0
100 gm. diet	3	♂	36	17	114	11	64	1.5	24.0
	3	♀	34	22	120	12	66	1	27.7
	4	♂	38	22	117	12	69	3	33.8
		Av.	38	20	114	11	65	2.0	29.0
3. Sodium citrate	1	♀	34	12	99	4	56	3.5	34.3
0.04 mol. in	2	♂	44	28	135	4	60	4	35.4
100 gm. diet	3	♂	42	20	105	10	63	3	31.7
	3	♀	36	22	116	6	57	2.5	32.5
	4	♂	43	19	116	2	49	4	33.0
		Av.	40	20	114	5	57	3.4	33.4
4. Citric acid	1	♀	34	20	114	2	44	4	32.0
0.02 mol. plus	2	♂	40	23	123	11	65	3.5	32.2
sodium citrate	2	♀	42	23	132	9	60	3.5	37.3
0.02 mol. in	3	♂	40	22	123	10	65	3	33.3
100 gm. diet	4	♂	35	19	113	11	54	3	36.4
	4	♀	35	17	103	14	50	3.5	29.6
		Av.	38	21	118	10	56	3.4	33.5
5. Citric acid	1	♂	35	19	106	8	47	3.5	28.9
0.03 mol. plus	2	♂	42	16	112	8	63	4	37.9
sodium citrate	2	♀	40	20	102	8	64	4	39.6
0.02 mol. in	3	♂	40	22	123	10	63	3.5	32.4
100 gm. diet	4	♀	35	23	110	5	49	4	38.5
		Av.	38	20	111	8	57	3.8	35.5
6. Lactic acid	1	♂	36	20	108	10	47	1	22.5
0.04 mol. in	1	♀	38	24	125	8	54	1.5	28.4
100 gm. diet	2	♂	40	20	113	2	58	2.5	34.0
	3	♀	38	22	127	4	55	R	25.3
	4	♀	34	18	103	6	52	1	25.0
		Av.	37	21	115	6	53	1.2	27.0
Experiment II									
1. None (controls)	5	♂	42	20	119	10	92	R	25.0
	6	♀	48	22	162	22	96	R	29.2
	6	♂	45	29	179	18	100	R	26.0
	7	♀	50	20	159	12	82	R	21.0
		Av.	46	23	155	16	93	R	25.3
2. Potassium citrate	5	♂	43	20	123	1	77	3	35.1
0.04 mol. in	5	♂	48	24	155	4	105	3.5	39.9
100 gm. diet	6	♀	58	20	179	12	94	3.5	41.0
	7	♂	52	18	152	10	93	4	38.6
	7	♀	43	19	159	8	78	3	36.1
		Av.	49	20	154	7	89	3.4	38.1
3. Citric acid	5	♀	42	19	134	7	98	3	37.8
0.02 mol. plus	5	♂	50	21	146	9	104	3.5	41.4
potassium citrate	6	♂	48	24	169	18	110	4	46.1
0.02 mol. in	6	♀	46	28	172	14	100	3	38.6
100 gm. diet	7	♂	53	19	157	13	106	4	41.4
		Av.	48	22	156	12	104	3.5	41.1
4. Citric acid	5[1]	♂	46[1]	22[1]	133[1]	—8[1]	51[1]	4[1]	31.4[1]
0.03 mol. plus	5	♀	42	24	145	2	93	3	39.5
potassium citrate	6	♂	52	23	166	13	95	4	43.0
0.02 mol. in	7	♂	50	22	165	10	106	4	36.8
100 gm. diet	7	♀	50	20	160	10	85	4	38.9
		Av.	49	22	159	9	95	3.8	39.6

[1]Omitted in average since rat lost 8 gm. and did not eat at least 2 gm. every day during the experimental period (see U.S. Pharmacopeia XI).

3332:1 or over so that they are truly significant. The use of 0.03 mol. citric acid plus 0.02 mol. sodium citrate, equivalent to the proportion of citric acid-sodium citrate used by Shohl, did not significantly increase the improvement in calcification over the values on the lower concentration of citrates when at least 0.02 mol. of the citrate was given as salt. Therefore no tests were made by us of the higher concentration of citrate as either citric acid or sodium citrate alone. The odds were 57:1 that these values were more significant than the values found when citric acid alone was used. Lactic acid at 0.04 mol. level showed only slight improvement as measured by either line test or bone ash, and the results were not statistically significant.

In experiment II the addition of potassium citrate (0.04 mol. per 100 gm. basal ration), citric acid 0.02 mol. plus potassium citrate 0.02 mol., and citric acid 0.03 mol. plus potassium citrate 0.02 mol. all gave very satisfactory results; there were no line tests below 3 + cure and no bone ash values below 35%. The slightly higher line test in the citric acid 0.03 mol. plus potassium citrate 0.02 mol. did not seem significant and the bone ash values were somewhat lower. The odds for the significance of the improvement in bone ash values by the addition of potassium citrate or citric acid—potassium citrate mixtures were all beyond the limits of table IX in Love's text ('37), but the ratios were $>$ 3332:1 for the first two supplements and $>$ 1999:1 when the higher concentration of citric acid was used. The differences in the mean values for these three supplements are not statistically signicant.

The results of experiments I and II are not entirely comparable due to litter differences and possibly to differences in initial weights and food intakes, but statistical analysis of the values has shown that potassium citrate was more effective either alone or in combination with equal parts of citric acid than corresponding amounts of sodium citrate. The odds were 249:1 that the improvement was not due to chance using the values for potassium and sodium citrates alone and 712:1

using the mixtures of 0.02 mol. of acid and salt. The differences in values for 0.03 mol. acid plus 0.02 mol. salt were not significant, probably due to the wide variation found among the animals on the citric acid-sodium citrate supplement.

DISCUSSION

Examination of the data presented will show that the variation in food intake among the animals given any specific supplement was greater than the variation in food intake among the animals on different supplements in that experiment. Also there is no evident correlation between the bone ash values and sex, gain in weight or food intake. There seems to be a litter difference, e.g., it will be noted that in experiment I the animal showing the highest bone ash value in each group is from litter 2, except the citric acid 0.04 mol. supplement; in experiment II the animal showing the highest bone ash value in every group is from litter 6; and seven out of the eight animals from each of these litters show bone ash values above the average for their specific supplement.

Before discussing the line test and bone ash results on the different supplements in these experiments, attention should be called to the results on bone ash values on rachitogenic diets reported by Zucker ('36). He found a mean value of 30% ash for rachitic rats at 3 weeks of age, the actual values varying with season, rat strain, etc. Using the 10-day curative experiments used in vitamin D assays (U.S.P. technic) 2 + cures gave a bone ash mean value of 34%. Using fifty or one hundred times the dose required to produce the 2 + cure did not raise the value above 40% although a 4 + cure was found by line test. He also found that the variations in bone ash values increase the further they were removed from the normal. The results of our experiments were comparable to his, mean values of 24.8 to 25.3% were found for control rats, 29% for a 2 + cure, and from 33.4 to 41.1% for 3 to 4 + cure. Only five rats from any group showed values over 40%.

Attention might be called to the line test and corresponding bone ash values given by Shohl ('37). His table 2 shows that with diets S and K, slightly basic basal diets, the addition of the citric acid plus sodium citrate cured the rickets (by histological and x-ray diagnosis) but materially lowered the bone ash values, in diet S by 4%, in diet K by 9%. In the slightly acid diets C and D the 'rickets' was cured with practically no change in bone ash values. Again in table 4 the feeding of lactates gave a slightly better bone ash than the feeding of tartrates, but his decision of cure for the tartrates and no cure for the lactates was again based on the histological and x-ray diagnosis. It would seem that the level of bone ash is more clearly diagnostic of cure than change in the calcification in the epiphyseal line, since improvement in bone ash indicates improved calcification throughout the bone and 'cure of rickets' with continuance of osteoporosis does not materially improve the condition of the animal. Thus from his bone ash values in table 4 the citrates alone have produced normal bones, and in table 2 the bone ash values when citrates were fed were normal only in the case of 8:1 or 4:1 Ca:P ration on diets Y and St.

Our results agree in part with those of Shohl in that lactic acid did not significantly increase the calcification of the femur, and that citric acid produced less significant results alone than when fed as citric acid-sodium citrate mixtures, but they disagree in the amount of citrate ion required and in the fact that in our experiments sodium citrate produced as effective calcification as the citric acid-sodium citrate mixtures. Hamilton and Dewar ('37) reported no bone ash values in their paper, so that the results are not really comparable to ours. However, it might be mentioned that on the basis of line tests and x-ray measurements they found citric acid and sodium citrate about equally effective in producing cures, whereas we found a definite superiority of the salt on the basis of bone ash values. Results on the use of potassium citrate have not been previously reported.

No attempt has been made to explain where or how the citrate acts to increase the calcification, nor why the potassium salt is more effective than the sodium salt. pH studies on the intestinal tracts of these rats indicated that variations there were due more to individual differences than to differences in diet. Obviously there is much more work to be done on this problem, but since circumstances prevent further work on it by either of us at this time, it seemed advisable to report the progress that has been made.

SUMMARY AND CONCLUSIONS

1. The diet used was the Steenbock-Black rachitogenic diet 2965. To this was added lactic acid or citric acid and/or its salts primarily at the level of 0.04 mol. of supplement per 100 gm. basal ration.

2. The increase in bone ash values for the various supplements showed approximately the following averages: lactic acid 2%, citric acid 4%, sodium citrate 9%, citric acid-sodium citrate mixtures 9 to 11%, potassium citrate 13%, citric acid-potassium citrate mixtures 14 to 16%.

3. The effectiveness of citrates in improving the calcification in rats on a rachitogenic diet was confirmed, but the need of citric acid—citrate mixtures for the improvement was not confirmed.

4. The greater effectiveness of potassium citrate as calcifying agent was indicated.

5. The calcifying effect of citrates appeared to be unrelated to sex, gain in weight or food intake.

It is a pleasure to acknowledge the kindness of Dr. F. C. Koch of The University of Chicago in allowing us to use the facilities of his laboratories for the bone ash determinations.

LITERATURE CITED

GUBNER, R. 1937 The metabolic background of rickets. New Eng. J. of Med., vol. 216, p. 879.

HAMILTON, B., AND M. M. DEWAR 1937 Effect of citrate and tartrate on experimental rickets. Am. J. Dis. Children, vol. 54, p. 548.

HESS, A. F., AND M. J. MATZNER 1924 Value of milk acidified with lemon juice; its combination with egg yolk to add the antirachitic factor. J. Am. Med. Assoc., vol. 82, p. 1604.

LACHAT, L. L. 1935 Report on technic and details of biological methods, vitamin D carriers. J. Assoc. Off. Agr. Chem., vol. 18, p. 357.

LOVE, H. H. 1937 Application of statistical methods to agricultural research, The Commercial Press, Limited, Shanghai.

MCCOLLUM, E. V., N. SIMMONDS, P. G. SHIPLEY AND E. A. PARK 1922 Studies on experimental rickets: a delicate biologic test for calcium depositing substances. J. Biol. Chem., vol. 51, p. 41.

MORRIS, N., F. J. FORD AND S. GRAHAM 1936 The role of acidosis and phosphate retention in the pathogenesis of rickets and rachitic tetany of infants. Acta Ped., vol. 18, p. 50.

SCHMIDT, C. L. A., AND D. M. GREENBERG 1935 Occurrence, transport and regulation of calcium, magnesium and phosphorus in the animal organism. Physiol. Rev., vol. 15, p. 297.

SHOHL, A. T. 1937 The effect of the acid-base content of the diet upon the production and cure of rickets with special reference to citrates. J. Nutrition, vol. 14, p. 69.

STEENBOCK, H., AND A. BLACK 1925 Fat soluble vitamins XXIII. J. Biol. Chem., vol. 64, p. 263.

WEISSENBERG, H. 1928 Die Zitronensäurevollmilch in ihrer Bedeutung für die Bekämpfung von Saüglingssterblichkeit und Rachitis. Arch. für Kinderh., Bd. 84, S. 200.

ZUCKER, T. F. 1936 Bone ash in prevention and healing of experimental rat rickets. Science, vol. 84, p. 162.

THE UTILIZATION OF THE CALCIUM OF MILK BY PRE-SCHOOL CHILDREN [1, 2]

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Of all the foods commonly used in the American dietary, milk has long been recognized as being the most important source of calcium. Yet, the extent to which milk calcium is available for growth and for the maintenance of the physiological processes of man has received only scant attention. In estimating an individual's dietary requirement for calcium, it is as necessary to know how well he utilizes the calcium of the foods which he eats as it is to know the actual calcium content of those foods. Until this information is available for milk—and for other foods, too—the value of milk as a source of calcium in the dietary must rest on an insecure basis.

A few investigators have attempted to determine the availability of the calcium of milk, but their studies have been comparisons of milk and other calcium-rich foods or of milk and milk products. In those studies fluid milk has been used as the standard of reference, and the data have been reported in terms of comparative values. Thus, a similarity in the availability of the calcium of American cheese and that of

[1] A portion of these data was presented before the Division of Biological Chemistry of the American Chemical Society in Rochester, 1937, and before the American Home Economics Association in Pittsburgh, 1938.

[2] Aided by a grant from the American Dry Milk Institute.

[3] A portion of the data obtained on the boys was presented by Elizabeth Jensen in partial fulfillment for the degree of master of science at the University of Illinois, June, 1938.

pasteurized milk was reported by Mallon, Johnson and Darby ('32); Kramer, Potter and Gillum ('31) found raw milk and ice cream made with condensed milk to be equally valuable as sources of calcium. Conversely, a slightly superior value was ascribed to the calcium of evaporated milk over that of pasteurized milk by Willard and Blunt ('27). Likewise, raw milk has a greater comparative value than has dried milk or pasteurized milk, according to Kramer, Latzke and Shaw ('28).

The manner in which the above studies were conducted does not permit an interpretation of the data in terms of absolute values for utilization. According to Mitchell and McClure ('37).

> . . . . the proper method of measuring the degree of utilization of minerals in a feed or ration may be more of a problem than is generally realized. The ordinary method of estimating this utilization is to express the retention of the mineral as a percentage of the intake. However, if there is a maintenance requirement for a mineral, it is not represented in the retention as ordinarily computed, so that a comparison of the latter with mineral intake credits the intake with only part of its nutritive effect and hence underrates its utilization. This underrating becomes the more serious the lower the intake of the mineral. The most practical measurement of the utilization of the mineral content of a feed would seem to be that secured at a level of intake of the particular element studied that just suffices (or that just barely does not suffice) to cover the integrated requirements of the animal for it.

The study reported in this paper constitutes an attempt to determine the extent of the utilization of the calcium of milk by pre-school children. A procedure has been adopted which eliminates the effect of a possible maintenance requirement for calcium. This has been accomplished by determining calcium balances at two levels of intake, both of which gave positive balances and therefore were above the maintenance requirement but neither of which was in excess of the minimum requirement for the maximum retention of calcium by the subjects. In computing percentage utilization, use was made

of the increment in calcium retention in relation to the increment in intake between the two levels, rather than (as is commonly done) the total retention in relation to the total intake during one period. This procedure is illustrated by the following formula where 'a' represents a period of high intake and 'b' signifies one of lower intake.

$$\frac{\text{Retention for period a} - \text{retention for period b}}{\text{Intake for period a} - \text{intake for period b}} \times 100 = \text{per cent utilization}$$

This formula is advantageous in that it makes possible a differentiation between retention and utilization and provides for a quantitative, absolute measure of the utilization of the calcium in a given substance, e.g., milk. The only assumption made is that the maintenance requirement is not influenced by the change in the quantity of calcium ingested. Obviously, the formula cannot be applied if the substance used to raise the calcium level causes a change in the utilization of the calcium in the basal diet.

EXPERIMENTAL

The data for this publication were taken from a study of the calcium metabolism of five pre-school girls (Outhouse, Kinsman et al., '39) and from a similar study now in progress on six pre-school boys. The ages, weights and heights and the comparisons of these with Grandprey's ('33) modification of Woodbury's standards are given in table 1 for the boys

TABLE 1

The ages, weights and heights of the subjects and the comparisons of these with Grandprey's ('35) standards

SUBJECT	AGE [1]		WEIGHT [1]	HEIGHT [1]	LOCUS OF MEASUREMENTS		
	Years	Months			Weight-Age	Height-Age	Weight-Height
			kg.	*cm.*	%	%	%
R	2	11	16	99	90	Above 90	75
W	4	0	16	100	35	50	75
C	5	1	20	111	90	90	80
G	5	6	20	115	80	85	70
Br	5	11	23	116	Above 90	85	Above 90
Jw	6	0	20	117	75	85	50

[1] Taken at sixteenth week of experiment.

and in the above publication for the girls. All the subjects were maintained on the regimen described in the aforementioned paper. In both studies calcium balances were determined for every 7 days during the entire experimental period. The time intervals, designated in the reports as periods II, III, IV, etc., varied in length from 28 to 56 days. From period to period, changes in the calcium level were brought about by increasing the quantity of the supplement of milk solids.[4] These increments in calcium were planned to give significant differences in retentions and at the same time, to keep the highest level of intake below the minimum requirement for maximum retention. The experimental procedures adopted for the collection of metabolic materials, for sampling and for analysis for calcium were the same for both the boys and the girls.

A slight difference between the two studies existed wherein the levels of calcium intake were concerned. At the lower level, the girls received 370 mg. of calcium daily whereas the boys received 340 mg. For the higher levels, the girls were given 615 mg.; and, since it was found that this quantity was very close to the minimum requirements of the girls, a smaller quantity (555 mg.) was given to the boys. At the subsequent higher levels of calcium intake, additional values for percentage utilization of the calcium in milk were computable for some of the boys due to the fact that the quantity of calcium ingested at the higher levels was not in excess of their minimum requirements. The data for the various periods with reference to the length of the periods, the average daily intakes of calcium and the amount of calcium contributed by milk solids are presented in table 2.

RESULTS

Only those metabolic data which are pertinent to the subject of the utilization and retention of calcium are presented in this paper (table 3). The actual intakes and retentions of calcium by the individual subjects during the various periods

[4] Skim milk powder the calcium content of the various lots of which ranged from 123 to 135 mg. per gram.

are given in terms of the average daily quantities involved. From the data for retentions, it is apparent that during period IV the 880 mg. level of calcium intake for the girls was in excess of their minimum requirement. For the boys, the level of intake (704 mg. as a daily average) during this period did not give maximum retentions as is indicated by the greater retentions during the subsequent period of higher intake.

TABLE 2

Intakes of calcium, amounts of calcium contributed by milk solids and total calcium retentions during the various periods of the experiment

PERIOD		CALCIUM INTAKE (AVERAGE DAILY)		CALCIUM RETENTIONS (AVERAGE DAILY)
Number	Length	Total	Contributed by milk solids	
		Girls		
	days	mg.	mg.	mg.
1	77	1815	258	126
2	56	370	258	76
3	35	615	516	122
4	28	880	774	112
		Boys		
1	91	1600	1518	140
2	42	340	231	56
3	35	555	444	100
4	28	704	590	125

Values for the percentage utilization of the calcium of milk have been calculated by the formula presented in the introduction of this paper, using the intake and retention values of periods II and III for the girls and of periods II and III and periods II and IV for the boys. The single values obtained for girls B, M, J, P and S were 18, 14, 22, 25 and 16%, respectively; they averaged 19%. The mean values for boys R, W, C, G, Br and Jw were 18, 21, 20, 13, 19 and 27%, respectively, with a mean value of 20%. The mean value for the eleven subjects was 19% with a standard deviation of 4.5 and a coefficient of variation of 23%. The maximum variation of the values for the subjects was 37% above and 34%

TABLE 3

Total intake and retention of calcium and the utilization of calcium of milk by pre-school children

SUBJECT	INTAKE—AVERAGE DAILY DURING PERIODS				RETENTION								UTILIZATION—COMPUTED FROM DATA FOR PERIODS		
					Average daily during periods				Relation to intake during periods						
	II	III	IV	V	II	III	IV	V	II	III	IV	Average	II & III	II & IV	Average
Girls															
	mg.	*mg.*	*mg.*	*mg*	*mg.*	*mg.*	*mg.*	*mg.*	%	%	%	%	%	%	%
B	357	604	862		72	117	85		20.2	19.4		19.8	18.2		
M	357	604	865		50	84	108		14.0	13.9		13.9	13.8		
J	378	615	900		94	145	118		24.8	23.5		24.1	21.5		
P	371	617	875		80	142	154		21.6	23.0		22.3	25.2		
S	384	640	892		84	124	94		21.9	19.3		20.6	15.6		
Mean	369	616	879		76	122	112		20.5	19.8		20.1	18.9		
Boys															
R	328	554	692	891	63	94	143	158	19.2	16.9	20.6	18.9	13.7	21.9	17.8
W	329	548	703	899	68	116	145	183	20.7	21.1	20.6	20.8	21.0	22.1	21.5
C	347	560	712	909	63	81[1]	136	145	18.1	(14.4)[1]	19.1	18.6	(18.4)[1]	20.0	20.0
G	343	567	720	915	53	83	98	126	15.4	14.6	13.6	14.5	13.4	11.9	12.7
Br	344	556	699	904	60	106	119	179	17.4	19.0	17.0	17.8	21.7	16.6	19.1
Jw	345	545	697	905	64	119	110[1]	135	18.6	21.8	(15.7)[1]	20.2	27.5	(13.2)[1]	27.5
Mean	339	555	704	904	62	103	125	154	18.3	18.0	18.0	18.5	19.5	18.5	19.8
Girls and Boys															
Mean									19.2	19.2		19.2			19.3

[1] Data not included in calculations since retentions were obviously too low.

below the mean. This variation may appear to be great, yet from a biological standpoint the figures are all of the same order of magnitude and indicate poor utilization of the calcium of milk by pre-school children.

The data for retention of calcium expressed as the percentage of the intake (i.e., percentage retention) for the girls for periods II and III and for the boys for periods II, III and IV are also given in table 3. For girls B, M, J, P and S, the average percentage retentions for periods II and III were 20, 14, 24, 22 and 20, respectively, averaging 20%. For boys R, W, C, G, Br and Jw the average values for periods II, III and IV were 19, 21, 19, 15, 18 and 20, respectively, and averaged 18%. The range and the average of the values for the girls did not differ significantly from those for the boys. The total average for the eleven subjects was 19%.

DISCUSSION

The fact that only from one-fifth to one-fourth of the calcium of milk is utilized and retained by children may seem surprising. However, earlier reports contain data from which the same conclusion could have been drawn. In the Sherman and Hawley study ('22) in which the milk intake of two preschool girls (subjects M.O. and K.C.) was increased and decreased at the rate of 250 cc. daily, the percentage utilization values, computed from increments in intake and in retention at intakes ranging from 380 to 950 mg., averaged 20. The percentage retentions, calculated from the same data at the various levels of intake, averaged 29. In Petrunkina's ('34) study on children from 6 to 9 years old, the percentage utilization values (computable for nine of the subjects) ranged from 8 to 50 with a mean of 19%; the percentage retentions averaged 16. These are the only papers found in the literature which permit the computation of percentage utilization. However, considerable data concerning percentage retentions may be obtained from several other studies in which the calcium intakes may not have been in excess of the subjects' requirements. The values, which are inherent in the data of the

respective authors, are as follows: 18% for seventeen healthy pre-school children studied by Daniels and co-workers ('34, '35); 23% for Porter-Levin's ('32–33) two healthy children; 20% for six healthy subjects (ranging in age from 5 to 7 years) studied by Bonner et al. ('38); and 22% for nineteen normal and underweight children studied by Wang, Kaucher and Frank ('28). The average for the forty-six subjects who were under 7 years of age is 21%. This figure compares favorably with the percentage retention value reported for the eleven children in this study. Both are considerably less than half the value of 50% which was assumed by Leitch ('37) for the retention of calcium from a 'suitably balanced mixed diet.'

The data secured in this study have a direct bearing upon the fundamental question as to whether or not the maintenance requirement of calcium, as observed in the adult animal, may not be considerably or entirely suppressed in the growing organism. There is considerable evidence in the literature in favor of this possibility. Extremely low excretions of calcium in essentially normal, growing boys subsisting on low-calcium diets were reported by Albright, Bauer, Ropes and Aub ('29) and by Farquharson, Salter, Tibbetts and Aub ('31). Furthermore, Aub, Tibbetts and McLean ('37) observed similar low excretions in a growing boy following a parathyroidectomy. They have suggested that ". . . . a new mechanism had obviously come into play; and both urinary and fecal calcium were being avidly retained in spite of higher blood levels above the so-called kidney threshold. . . ." This 'new mechanism' may be merely a suppression of endogenous losses of calcium brought about by the growing skeleton's intensified demand for calcium and by a simultaneous, inadequate dietary supply. Ellis and Mitchell ('33) showed that growing rats on a severely restricted calcium intake may deposit in their bodies 98% of the calcium consumed, leaving a maximum of only 2% of the intake available for replacement of endogenous losses (assuming a complete alimentary utilization). This efficient utilization of dietary calcium at low levels of

intake was confirmed by Fairbanks and Mitchell ('36); they also observed that when a high-calcium diet was fed, variable rates of growth (from 2.38 to 5.08 gm. per day) were associated with constant percentage retentions of dietary calcium. If dietary calcium had replaced endogenous losses, greater percentage retentions of calcium would have been observed during periods of rapid growth and increased food intake, since a greater proportion of the consumed calcium would have been available for growth.

If an appreciable portion of the dietary calcium consumed by the pre-school children of this study had been used to replace endogenous calcium losses (i.e., if an appreciable maintenance requirement of calcium had existed) two effects would have been observed. In the first place, the values for percentage retention of calcium would be less than the values for percentage utilization, since, as was explained before, the latter values are based on intakes which were above the maintenance requirement. Contrary to this expectation, there was no consistent difference between the values for the percentage retention of calcium and those for the percentage utilization. These respective values for each of the eleven subjects were 20 and 18, 14 and 14, 24 and 22, 22 and 25, 20 and 16, 19 and 18, 21 and 21, 19 and 20, 15 and 13, 18 and 19, and 20 and 27, with respective averages of 19.3 and 19.2%. These results are consistent with the hypothesis that the endogenous losses of calcium were entirely suppressed in these children.

The other effect of an appreciable requirement for calcium for maintenance would be an increase in the percentage retention with increasing intake of calcium up to the point of maximum retention. However, in only four of the eleven children was a greater percentage retention of calcium observed on the higher calcium level, and since the averages for the eleven subjects during periods II and III are identical (19.2%), it is safe to suggest again that no appreciable portion of the dietary calcium could have been used in replacing the endogenous calcium lost by way of the intestinal tract and kidneys.

The above reasoning implies that the calcium of the basal foods (which supply approximately 30% of the total calcium during period II) is utilized to an extent no greater than is that of milk calcium, i.e., 20%. If the calcium of these foods were utilized to a greater degree, then a maintenance requirement for calcium must be assumed in order to account for the balance data secured. A utilization of 100% of the basal-food calcium would be consistent with an average daily maintenance requirement (endogenous loss) of 5 mg. per kilogram of body weight. Even if the non-milk calcium had been utilized completely, the 10-mg.-per-kilogram value used by Leitch ('37) in her estimation of children's requirements for dietary calcium would have resulted in negative balances for all six boys during period II and for four boys during period III, and also would have resulted in much lower retentions for the girls than were actually observed. Furthermore, if these children had had a maintenance requirement no greater than that computed by Mitchell ('38) from available information on adults (i.e., 3.1 mg. per kilogram) they would have had to utilize, as an average, 64% of the basal-food calcium. However, there is no evidence in the literature of superior availability of the calcium of ordinary foods over that of milk calcium. On the contrary, experiments have shown that human subjects are able to utilize the calcium of milk just as well as, if not better than, that of other foods. If this is true, then the contention that the children did not have an appreciable maintenance requirement for calcium may be accepted.

Ellis and Mitchell ('33) have portrayed the endogenous metabolism of calcium in the growing animal in the following words:

> Calcium ions liberated in the course of catabolism from functional combinations in the tissues and body fluids are probably just as available for the reconstruction of such combinations as are calcium ions picked up from the intestinal tract. In the young growing animal these 'used' ions as well as the calcium ions coming directly from the alimentary tract may be absorbed and retained by the developing bones and the growing tissues at such a rapid rate that, at low levels of calcium

feeding the threshold of excretion by the kidneys and the intestines is never reached.

If this is a true picture of the situation, the re-utilization of calcium ions liberated in the endogenous catabolism of the tissues may not occur at liberal levels of calcium ingestion, or at least not to the same extent as it would at levels commensurate with the requirements for maximum growth. During periods of generous intake, endogenous losses of calcium would appear and a maintenance requirement for calcium, in the usual sense of the term, would develop.

If subsequent research should prove the above hypothesis and establish its general applicability to growing children, information of considerable practical value should result. One of the possible deductions might be that a value for percentage retention would be indicative of the extent to which the growing child can utilize the calcium of the usual mixed diet, or of how well he can utilize the calcium of a specific food when that food contributes the greater part of the total calcium intake. Thus, the values for percentage retention heretofore reported for those children who were fed submaximal levels of calcium could correctly be interpreted as percentage utilization. Another obvious conclusion would be that, in estimating a child's requirement for calcium, provision need be made only for the calcium requirements of growth.

The limited ability of the pre-school child to utilize milk calcium is in marked contrast with that reported by Ellis and Mitchell ('33) in their studies on rats. They reported retention values as high as 98% for this species. In that study, as in the present one, the experiments were conducted in such a manner as would insure maximum values for utilization in each instance, i.e., the subjects were taken during the period of growth, and the level of calcium at which they were fed was not in excess of their minimum requirements for maximum retention of calcium. The striking disparity in the results obtained indicates a tremendous difference in these two species and provides an excellent illustration of the frequently unappreciated fact that, quantitatively, rats and human subjects

do not necessarily react similarly. This finding points to the need for more extensive studies on human subjects before satisfactory dietary standards can be promulgated.

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LITERATURE CITED

ALBRIGHT, F., W. BAUER, M. ROPES AND J. C. AUB 1929 Studies of calcium and phosphorus metabolism. IV. The effect of the parathyroid hormone. J. Clin. Invest., vol. 7, p. 139

AUB, J. C., D. M. TIBBETTS AND R. MCLEAN 1937 The influence of parathyroid hormone, urea, sodium chloride, fat and of intestinal activity upon calcium balance. J. Nutrition, vol. 13, p. 635.

BONNER, P., F. C. HUMMEL, M. F. BATES, J. HORTON, H. A. HUNSCHER AND I. G. MACY 1938 The influence of a daily serving of spinach or its equivalent in oxalic acid upon the mineral utilization of children. J. Pediatrics, vol. 12, p. 188.

DANIELS, A. L., M. K. HUTTON, E. KNOTT, G. EVERSON AND O. WRIGHT 1934 Relation of ingestion of milk to calcium metabolism in children. Am. J. Dis. Child., vol. 47, p. 499.

DANIELS, A. L., M. K. HUTTON, E. M. KNOTT, O. E. WRIGHT AND M FORMAN 1935 Calcium and phosphorus needs of pre-school children. J. Nutrition, vol. 10, p. 373.

ELLIS, M., AND H. H. MITCHELL 1933 The effect of the pasteurization of milk on the utilization of its calcium for growth in the rat. Am. J. Physiol., vol. 104, p. 1.

FAIRBANKS, B. W., AND H. H. MITCHELL 1936 The relation between calcium retention and the store of calcium in the body, with particular reference to the determination of calcium requirements. J. Nutrition, vol. 11, p. 551.

FARQUHARSON, R F., W. T. SALTER, D. M. TIBBETTS AND J. C AUB 1931 Studies of calcium and phosphorus metabolism. XII. The effect of the ingestion of acid-producing substances. J. Clin. Invest., vol. 10, p. 221.

GRANDPREY, M. B. 1933 Range of variability in weight and height of children under six years of age. Child Devel., vol. 4, p. 26.

KRAMER, M. M., E. LATZKE AND M. M. SHAW 1928 A comparison of raw, pasteurized, evaporated and dried milks as sources of calcium and phosphorus for the human subject. J. Biol. Chem., vol. 79, p. 283.

KRAMER, M. M., M. T. POTTER AND I. GILLUM 1931 Utilization by normal adult subjects of the calcium and phosphorus in raw milk and ice cream. J. Nutrition, vol. 4, p. 105.

LEITCH, I. 1937 The determination of the calcium requirements of man. Nutr. Abs. and Rev., vol. 6, p. 553.

MALLON, M. G., L. M. JOHNSON AND C. R. DARBY 1932 A study of the calcium retention on a diet containing American Cheddar cheese. J. Nutrition, vol. 5, p. 121.

MITCHELL, H. H. 1938 The dietary requirement of calcium and its significance. Monograph Series entitled Nutrition, edited by E. F. Terroine.

MITCHELL, H. H., AND F. J. MCCLURE 1937 Mineral nutrition of farm animals. Bull. Nat. Research Council. No. 99, p. 51.

OUTHOUSE, J., G. M. KINSMAN, D. SHELDON, I. J. TWOMEY, J. SMITH AND H. H. MITCHELL 1939 The calcium requirement of five pre-school girls. J. Nutrition, vol. 17, p. 199.

PETRUNKINA, A. 1934 Über die Belanzen von Stickstoff, Kalk, Magnesium, Phosphor und Eisen bei Kindern von 7–8 Jahren. Z. Kinderheilk, Bd. 56, S. 219.

PORTER-LEVIN, T. 1932–1933 Calcium and phosphorus metabolism of normal pre-school children. I. On diets containing plain and irradiated cereals. J. Am. Dietetics Assoc., vol. 8, p. 482.

SHERMAN, H. C., AND E. HAWLEY 1922 Calcium and phosphorus metabolism in childhood. J. Biol. Chem., vol. 53, p. 375.

WANG, C. C., M. KAUCHER AND M. FRANK 1928 Metabolism of undernourished children. IV. Calcium metabolism. Am. J. Dis. Child., vol. 35, p. 856.

WILLARD, A. C., AND K. BLUNT 1927 A comparison of evaporated with pasteurized milk as a source of calcium, phosphorus and nitrogen. J. Biol. Chem., vol. 75, p. 251.

RELATIONSHIP BETWEEN CAROTENE, BLINDNESS DUE TO CONSTRICTION OF THE OPTIC NERVE, PAPILLARY EDEMA AND NYCTALOPIA IN CALVES [1]

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SEVEN FIGURES

(Received for publication January 11, 1939)

In 1935 Moore, Huffman and Duncan ('35) summarized the previous literature and reported on the results of observations of twenty-four cases of blindness of a nutritional type associated with a constriction of the optic nerve. The authors were reluctant to state that this blindness was due to vitamin A deficiency because of the bone changes involved, lack of corneal involvement and because no direct unimpeachable evidence was at hand to support such a statement. It was pointed out, however, that if vitamin A deficiency was responsible for an increased intracranial pressure the bony malformations might then be accounted for. Evidence of increased intracranial pressure is to be reported in this paper and resulted from the suggestion received in a paper reported by De Schweinitz and De Long ('34) who observed blindness and papillary edema in Guernsey calves associated with perivascular edema in the brain tissue. They suggested that the type of blindness was due to inheritance. However, the evidence submitted in this paper will show that a relationship exists between the condition reported by them and by Moore, Huffman and Duncan ('35).

[1] Journal article 346 (n.s.) Michigan Agricultural Experiment Station. From a portion of a thesis presented to the Graduate School of Michigan State College in partial fulfillment for the Ph.D. degree.

Mellanby ('38) has noted widespread nerve degeneration in young dogs fed diets deficient in vitamin A and rich in cereals. Complete deafness was present in some of the animals which he attributed to nerve degeneration due to an overgrowth of bone of the labyrinthine capsule. He expressed the belief that the degeneration of other nerves might be due to pressure caused by overgrowth of bone in the various foramina.

Guilbert and Hart ('35) established the relationship of night blindness and vitamin A deficiency in the bovine and by use of the night blind condition worked out the minimum requirements for vitamin A. In their paper they expressed the opinion that the blindness reported by Moore et al. ('35) was due to vitamin A deficiency but offered no direct evidence to support the belief. Kuhlman, Gallup and Weaver ('36) reported the development of xerophthalmia and a permanent type of blindness in calves fed a ration of beet pulp and cottonseed meal. Vitamin A supplements cured the xerophthalmia but had no effect on the permanent type of blindness. Phillips and Bohstedt ('38) report the preventive and remedial effects of carotene in oil on rabbits which developed a syndrome of symptoms similar to calves. The rabbits, however, failed to develop a constriction of the optic nerve and no ophthalmoscopic observations were reported.

Hale ('35) published data showing that vitamin A deficiency produced congenital anophthalmos and other abnormalities in swine.

EXPERIMENTAL

Subsequent to the previous report it was found that cod liver oil in which the vitamin A was destroyed by aeration at an elevated temperature did not prevent the constricted nerve or permanent type of blindness from developing in young calves. Another series of calves was used in which 'caritol' was diluted with cottonseed oil and then homogenized into skim milk so that the fat content of the remade milk was 2%. The carotene content of the milk was so adjusted that the

calves received one and two times the minimum requirement suggested by Guilbert and Hart ('35). The calves were started on the milk when 1 week to 10 days old. However, most of the calves died at an early age of pneumonia so that no data were obtained relative to blindness. It was then decided to raise the calves on whole milk and grain to about 90 days of age and then feed skim milk together with a low carotene grain ration consisting of 240 pounds barley, 180 pounds rolled oats, 180 pounds wheat bran, 60 pounds linseed oil meal and 8 pounds salt. This ration contained from 0.5 to 0.7 micrograms of carotene per gram so that the calves received 2 to 3 micrograms per pound of body weight from this source.

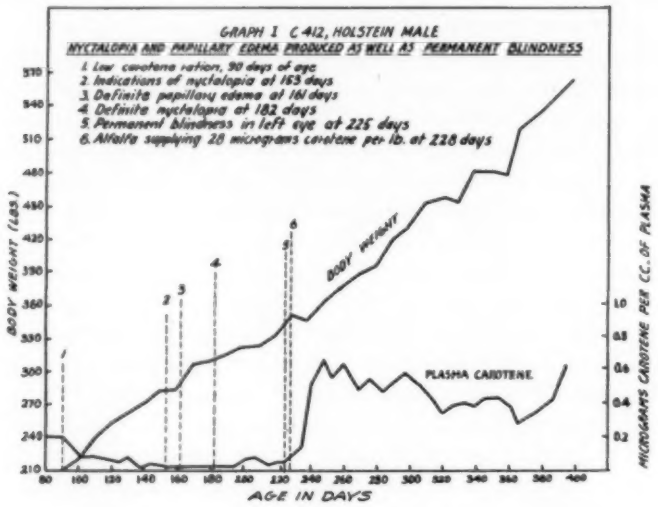
After the animals were placed on this ration, ophthalmoscopic examinations were made weekly. The calves were also tested for night blindness each week by attempting to run them into objects and watching their behavior in dim light, a method similar to that used by Guilbert and Hart ('35). Blood plasma carotene determinations were also made each week according to a method developed by the author ('38). Carotene extractions on the hays and feeds used were made according to the modification by Peterson et al. ('37) of the Guilbert method and the carotene determined by the use of a photoelectric colorimeter. The calves were weighed every 10 days at which time adjustments in the amounts of carotene to be fed were made.

The results obtained in three typical cases will be described in detail although observations have been made on many more animals.

Calf C412 was placed on the low carotene ration at 90 days of age, the principal results being shown in graph 1.

At 106 days of age, a folding of the lens capsule was noted on the posterior pole of the right eye and at this time it was observed that the tapetum lucidum of the retina of both eyes was quite bleached. At 161 days the nerve head of the right eye showed definite swelling and the nerve margins were entirely blurred as shown in figure 2. The nerve head showed

a choking of 1½ diopters. At 182 days definite night blindness was present although different degrees of night blindness had been observed after 153 days. Also at 182 days the left eye showed a definite edema with a choking of 0.5 to 1 D of the disc. It was also noted that some of the small vessels of the nerve head were quite dilated. At 203 days, the edema had increased considerably in the left eye so that there was a choking of 2 D. At this time also many of the capillaries and


GRAPH I C 412, HOLSTEIN MALE
NYCTALOPIA AND PAPILLARY EDEMA PRODUCED AS WELL AS PERMANENT BLINDNESS
1. Low carotene ration, 90 days of age
2. Indications of nyctalopia at 153 days
3. Definite papillary edema at 161 days
4. Definite nyctalopia at 182 days
5. Permanent blindness in left eye at 225 days
6. Alfalfa supplying 28 micrograms carotene per lb. at 228 days
BODY WEIGHT (LBS.)
BODY WEIGHT
PLASMA CAROTENE
MICROGRAMS CAROTENE PER CC. OF PLASMA
AGE IN DAYS

Graph 1.

small vessels at the edge of the nerve head showed considerable fusiform dilation. At 225 days the pupil of the left eye did not react to light stimulation and showed mydriasis. By means of covering each eye separately it was found that in daylight the calf was completely blind in the left eye but could still see well out of the right eye. However, in the dark the animal showed complete night blindness in the right eye. The

Fig. 1 Normal bovine fundus oculi.

Fig. 2 Fundus showing papillary edema and bleaching of the tapetum lucidum in vitamin A deficiency.

A. Rudolphy

A. Rudolphy

nerve head of the right eye showed edema and some congestion but none of the smaller vessels were dilated. Both eyes showed some exophthalmus.

At 228 days carotene was added in the form of alfalfa hay to the ration at a rate intended to supply 28 micrograms of carotene per pound of body weight in an attempt to preserve the sight of the right eye. The attempt was successful for at the end of 342 days the calf could still see. The sight of the left eye never returned due most likely to a constriction of the optic nerve where it passes through the optic foramen. At 290 days the tapetum lucidum of both eyes showed considerable orange yellow color. The nerve head of the right eye showed a choking of 2 diopters. The nerve head of the left eye looked quite smooth and was quite dark in color indicative of atrophic changes. The vessels of this eye also appeared considerably smaller than normal.

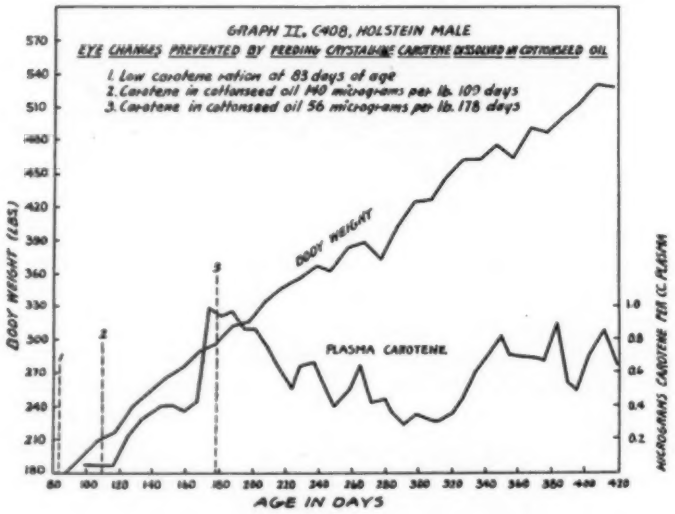
It will be noted from graph 1 that the addition of alfalfa caused an immediate rise in the plasma carotene of this calf.

Calf C408 was placed on the low carotene ration at 83 days of age and at 109 days crystalline carotene dissolved in cottonseed oil was added to the ration at the rate of 140 micrograms per pound. An immediate response in plasma carotene was noted as shown in graph 2. At 178 days the carotene level of the ration was dropped to 56 micrograms and there was an immediate drop in the level of carotene in the plasma. This animal at no time showed any indication of nyctalopia or evidence of papillary edema throughout the period of the experiment. There was some traumatic injury to the cornea and clouding of the anterior chamber as well as some slight hemorrhage on the retina of the right eye.

Calf C411 was placed on the low carotene ration at 90 days of age and at 128 days showed some indications of night blindness but no ophthalmoscopic alterations were observed except bleaching of the retina. Since it was the purpose of the experiment to prevent the development of ocular changes in calves, C408 and C411, the carotene was added immediately to the ration of the latter at the rate of 140 micrograms. At

188 days carotene feeding was discontinued for 10 days and resumed at the 56 microgram level. Throughout the experiment this calf showed no ocular changes.

The carotene[2] added to the ration of the last two calves, C408 and C411, was obtained in crystalline form. One gram was dissolved in 2 liters of cottonseed oil by placing the flask in hot running tap water for about 6 hours with frequent shaking.


GRAPH II. C408, HOLSTEIN MALE
EYE CHANGES PREVENTED BY FEEDING CRYSTALLINE CAROTENE DISSOLVED IN COTTONSEED OIL
1. Low carotene ration at 83 days of age
2. Carotene in cottonseed oil 140 micrograms per lb. 109 days
3. Carotene in cottonseed oil 56 micrograms per lb. 178 days
BODY WEIGHT
FLASMA CAROTENE
BODY WEIGHT (LBS.)
MICROGRAMS CAROTENE PER CC. PLASMA
AGE IN DAYS

Graph 2.

In addition to the observations on calf C412 papillary edema has been noted by the author in eighteen calves and eight mature cows on low vitamin A rations. It has also been observed in three calves at birth whose dams received low vitamin A rations. Nyctalopia has also been seen many times and could be cured in 8 to 10 days by feeding carotene in the form of alfalfa hay. Space does not permit reporting these cases in detail although the results with the larger group

[2] Purchased from S. M. A. Corporation.

have contributed to the ultimate conclusions reached in this paper.

DISCUSSION

The results obtained with calf C412 clearly show that the ration used in this experiment produced nyctaolpia, papillary edema and permanent blindness. The permanent type of blindness which developed in the left eye was prevented in the right eye by the addition of carotene in the form of alfalfa hay. It is quite certain that blindness would have developed in the right eye also had not carotene been administered since in all previous observations blindness has always become bilateral.

The results with calves C408 and C411 give indisputable evidence that crystalline carotene added to the ration prevented the development of nyctalopia, papillary edema and the permanent type of blindness due to constriction of the optic nerve. One must therefore admit that a deficiency of carotene permits these conditions to develop and that the permanent blindness reported by other investigators was probably due to a vitamin A deficiency.

It will be noted that no mention was made regarding the development of xerophthalmia or keratitis. None was observed in these studies. As a matter of fact, in the extensive experiments on vitamin A deficiency in cattle made at this station only a few very mild cases of keratitis have been noted. None have proceeded to the extreme stage of inflammatory reaction of the eye structures even though many animals have died from vitamin A deficiency. The reason for this is not clear. It seems possible that the absence of dust and perhaps certain bacteria in the barns was responsible. Attempts to infect a susceptible calf's eye with the exudate from a rat's eye which showed xerophthalmia were unsuccessful.

The cause of the constriction of the optic nerve is apparently due to bone pressure on the nerve as shown in figure 4 B. That the bony canal is altered in structure and becomes smaller

in this condition is also shown in figure 5. As stated previously it is difficult to associate vitamin A deficiency with any bony malformation but if evidence of increased intracranial pressure could be obtained it might explain the cause of the blindness. Such evidence seemed to be present since the nerve heads of these calves showed definite papillary edema as

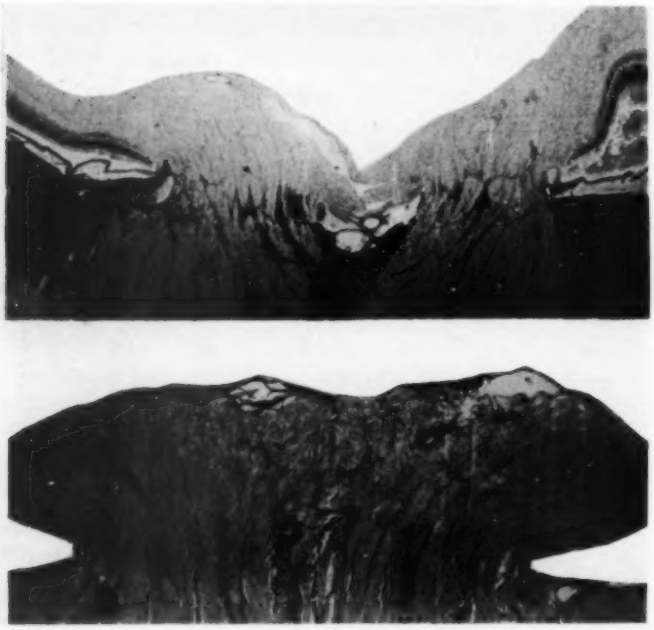


Fig. 3 A. Longitudinal section of a normal optic papilla. × 24. B. Longitudinal section optic papilla showing edema in vitamin A deficiency. × 24.

reported by De Schweinitz and De Long ('35) and shown in figures 2 and 3 B. Increased intracranial pressure exerted over a period of time could account for the displaced bone development since the brain itself lies directly above the optic canal. In a young calf the optic canal is very short, less than ¼ inch in length. In a mature animal it is 1½ inches

Fig. 4 A. Longitudinal section of a normal optic nerve and bony canal. × 16. B. Longitudinal section showing degeneration of the optic nerve and stenosis of the bony canal. × 16.

Fig. 5 Showing bony canals of blind and normal calves. C217 and C249 from blind calves showing stenosis; C280, normal.

in length so that over a 2½-year period there is considerable growth. Further evidence of this theory of constriction of the optic nerve is supported by the fact that mature cows develop papillary edema on vitamin A deficient rations but never show the permanent type of blindness. The reason they do not acquire the permanent type of blindness is because the bony canal has fully developed and calcified and therefore no abnormal growth anomaly results. Further evidence of increased intracranial pressure is shown by the condition of syncope, spasms and incoordination all of which are alleviated by vitamin A administration.

De Schweinitz and De Long ('35) did not note constriction of the optic nerve or a stenosis of the optic canal in their cases. In some cases examined by the author these changes were hardly discernible and unless great care is used in dissection they may be missed. It can be argued that very little pressure on the nerve is needed to produce a state of blindness. If the deficiency was corrected at this time the nerve and bony canal would probably show little change. However, if the deficiency was continued a stenosis of the canal and degeneration of the nerve would be produced. That the process can be arrested quite readily was demonstrated in the case of calf C412.

The particular cause of the increased intracranial pressure was not determined although De Schweinitz and De Long ('35) believed it was due to perivascular edema in the brain tissue and showed slides purportedly demonstrating a large perivascular spacing. In the present study it was impossible to secure sections from calves in which the perivascular spacing could not be considered an artifact even though several technics were tried. However, this does not alter the supposition that increased intracranial pressure was present.

Guilbert and Hart ('35) have expressed the belief that the degeneration of the optic nerve was due to vitamin A deficiency per se. However, in the light of present evidence this idea is hardly tenable since the constriction of the nerve always occurs where it passes through the optic canal. Histological

evidence of degeneration of nerves other than the optic have not been reported for the bovine in vitamin A deficiency. Furthermore, older cows in which the optic canal has formed do not develop these constrictions. Degeneration of any part of the optic nerve other than at the optic foramen in the calf could easily be accounted for by a decreased blood supply to the part, pressure due to increased intracranial pressure and the pinching effect of the malformed optic canal. Evidence of circulatory disturbance was found in one case in which there was a cystic development in Cloquet's Canal directly anterior to the entrance of nerve into the optic canal. Further evidence of disturbance in the region of the cranial cavity was noted by the quite frequent presence of cysts in the pituitary. The cysts usually occurred between the two lobes and were sometimes larger than the pituitary.

The development of papillary edema associated with vitamin A deficiency has not been reported in other animals. However, one might expect such to occur especially where a condition of syncope and incoordination exists. Incoordination in vitamin A deficiency may not be correlated with myelin degeneration according to work reported by Eveleth and Biester ('37) on pigs. It is entirely possible that the myelin degeneration noted in vitamin A deficiency by various investigators is due directly to increased intracranial pressure and not to vitamin A deficiency per se. Vitamin A may possibly function to regulate the intracranial pressure.

Blindness due to constriction of the optic nerve associated with stenosis of the optic canal has not been reported as occurring in other species and may be peculiar to the bovine because of the particular structure and relationship of cranial cavity and optic canals.

The development of nyctalopia in the calves confirms the observations reported by Guilbert and Hart ('35). Nyctalopia and papillary edema occur at about the same time during the deficiency period but do not necessarily develop simultaneously. The reason for the two conditions not developing at the same time is that they are due to different processes. The

papillary edema is probably due to development of intracranial pressure while the nyctalopia is due to depletion of the vitamin A content of the retina in which it is apparently necessary for the regeneration of visual purple. The development of both conditions, however, is due to vitamin A deficiency and they therefore may develop simultaneously also.

It appears that once the papillary edema develops it takes a considerable period for it to recede. In one calf which received carotene at a low level there was little or no recession even after 312 days of supplemental feeding. In another there was a recession of the edema after about 6 months of uninterrupted supplemental feeding. The same slow recession has been noted in mature cows even when they were turned to pasture.

Ophthalmoscopic observations offer a means of diagnosis of vitamin A deficiency in calves if it has proceeded to the point where nyctalopia has developed. Even after the curing of the night blindness ophthalmoscopic observation will inform the observer, by the presence of papillary edema, whether there has been a vitamin A deficiency during the previous 4 to 6 months. In mature cows, other retinal changes take place in vitamin A deficiency which will be reported later.

The amount of vitamin A reserves during the later stages of depletion could to some extent be gauged by the color of the tapetum lucidum of the retina. As depletion progressed the orange yellow color disappeared and was replaced by a pale light blue color as illustrated in figure 2 giving the retina a bleached appearance. The observations on retinal color must be made without exposure of the animals to bright light on the day of examination. It was the usual procedure therefore to leave the calves in the barn the day on which the ophthalmoscopic observations were to be made and to administer the mydriatic a short while before examination. On dark days it was usually unnecessary to administer a mydriatic.

The level of plasma carotene gave considerable information concerning the vitamin A reserves at the low levels at which

carotene was fed in these experiments. As a rule the carotene content of the blood plasma remained below about 0.13 micrograms per cubic centimeters for a considerable period of time before the development of nyctalopia and ocular changes. These low values were obtained for calves of the Holstein and Ayrshire breeds but would probably be higher for the Guernsey and Jersey breeds. The response to the administration of carotene was usually immediate except when the amount was small. C408 responded immediately to the administration of the crystalline carotene in cottonseed oil as shown in graph 2.

Studies are now in progress in which the actual cerebral spinal pressure measurements are made by spinal puncture and the use of a manometer. The pathological changes concerned with the increased pressure are also being studied.

SUMMARY AND CONCLUSIONS

1. Calves fed low carotene rations develop nyctalopia, papillary edema and a permanent type of blindness associated with a constriction of the optic nerve.
2. The development of these conditions can be prevented by supplementing the low carotene ration with crystalline carotene dissolved in cottonseed oil. Consequently these conditions appear to be a part of the syndrome of vitamin A deficiency in calves.
3. The development of the constriction of the optic nerve is associated with a stenosis of the optic canal, papillary edema and probably increased intracranial pressure.
4. The development of papillary edema and nyctalopia are separate processes but both are due to vitamin A deficiency.
5. Vitamin A deficiency in calves is usually not associated with the development of keratitis.
6. Vitamin A deficiency in calves is associated with a condition of syncope, most likely produced by increased intracranial pressure.
7. Vitamin A deficiency in calves may be diagnosed by the presence of both papillary edema and nyctalopia.

8. Papillary edema persists for a considerable period after feeding carotene.

9. Under proper conditions of observation bleaching of the tapetum lucidum of the retina is a good guide for the diagnosis of vitamin A deficiency in the bovine.

10. Plasma carotene determinations of the bovine also give an indication of the vitamin A reserve where the colorless vitamin A is not being fed and the carotene intake is somewhat limited.

The author wishes to express his appreciation to Dr. C. A. Hoppert of the chemistry department, Dr. E. T. Hallman of the pathology department, Dr. C. F. Huffman of the dairy department and to Dr. J. O. Wetzel of Lansing for their aid and interest in carrying out this work.

LITERATURE CITED

De Schweinitz, G. E., and Pierce De Long 1934 Blindness and papilledema in Guernsey calves. Arch. Ophthal., vol. 2, p. 194.

Eveleth, D. F., and H. E. Biester 1937 The significance of myelin sheath degeneration and its relation to incoordination. Am. J. Path., vol. 13, p. 257.

Kuhlman, A. H., W. D. Gallup and Earl Weaver 1936 Two types of blindness in cattle and their possible relation to vitamin deficiency. J. Dairy Sci., vol. 19, p. 433.

Guilbert, H. R., and G. H. Hart 1935 Minimum vitamin A requirement with particular reference to cattle. J. Nutrition, vol. 10, p 409.

Hale, Fred 1935 The relation of vitamin A to anophthalmos in pigs. Am. J. Ophthal., vol. 18, p. 1087.

Mellanby, E. 1938 Nerve degeneration and bone hypertrophy induced in young animals by diet. Proc. Physiol. Soc., J. Physiol., vol. 93, p. 42.

Moore, L. A., C. F. Huffman and C. W. Duncan 1935 Blindness in cattle associated with a constriction of the optic nerve and probably of nutritional origin. J. Nutrition, vol. 9, p. 533.

Moore, L. A. 1938 The determination of blood plasma carotene in the bovine with a photoelectric colorimeter. In process of publication in the Journal of Dairy Science.

Peterson, W. J., J. S. Hughes and H. F. Freeman 1937 Determination of carotene in forage. Ind. and Eng. Chem. Anal Ed., vol. 9, p. 71.

Phillips, P. H., and G. Bohstedt 1938 Studies on the effects of a bovine blindness producing ration upon rabbits. J. Nutrition, vol. 15, p. 309.

THE INFLUENCE OF DIFFERENT LEVELS OF MILK INTAKE ON THE ACID-BASE MINERAL BALANCES OF CHILDREN

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There is in the literature a dearth of scientific data on the complete acid-base mineral balance of children consuming a general mixed diet (Sawyer, Baumann and Stevens, '18; Cooley et al., '35). Likewise, little is known of the changes that take place in the acid-base mineral balances during childhood in response to any dietary change such as varying the levels of milk incorporated in a known mixed basal dietary. The present paper has been designed to contribute information on these and other factors pertinent to the chemistry of growth of children.

Growth and maintenance depend upon the intake, utilization and excretion of nitrogen and the individual acidic and basic elements. Each of these elements has specific and varied physiologic functions and contributes to the formation of a part of the body. One of the most important functions of the human organism as a whole, and one of the least understood, is the utilization of minerals in a constant attempt toward maintenance of the acid-base equilibrium. The problem is more complex during growth when equilibratory amounts of the acidic and basic mineral elements must be retained for the usual metabolic processes of maintenance, but, in addition to the acidic and basic minerals, a sufficient amount of base-forming minerals termed 'excess base' is required to take care of the growth needs in the enlargement of the body and its organs.

The state of the mineral metabolism of a subject may be expressed as his acid-base mineral balance, given in milli-equivalents (meq.) of 'excess base.' The fact that certain elements play primary roles in particular physiologic functions, such as calcium and phosphorus in bone formation, and phosphorus and sulfur in the synthesis of body protein, has led to an emphasis of their individual metabolism in recent years.

It has been stated (Greenwald, '22) that, for man, no relation between metabolism and acid and alkaline diets has been proved; nevertheless Shohl has pointed out (Shohl, '23) that alkaline diets are essential in infancy, where growth is rapid, and are strongly indicated in pregnancy and lactation. Despite the lack of clear-cut evidence of a requirement of excess amounts of acid or base in the food of children, milk is an alkaline food and in the growing child alkali in excess of acid must be stored (White House Conference on Child Health and Protection, '32). Inasmuch as milk yields an alkaline mineral residue containing significantly preponderant amounts of the basic elements (calcium, magnesium, sodium and potassium) over those of the acidic elements (phosphorus, chlorine and sulfur) and does change the percentage and proportions of the various elements in the entire diet, the present study is an attempt to evaluate the effect of different levels of milk intake on the acid-base mineral retentions of growing children over a considerable period of time.

In interpreting the changes in acid-base mineral balances of seven average, healthy children, ages 5 to 10 years, when ingesting different quantities of milk in their diet, it has been possible to consider factors of control and environment—factors of great importance and too often minimized, but adequately controlled during this study due to the facilities made available by the complete cooperation of the Methodist Children's Village, Detroit.[1]

[1] Details of the control, environment and care and handling of the children, together with the measurements taken and the methods of collecting and sampling the foods and excreta for the 5-day balance periods, are contained in succeeding publications (Hummel, Hunscher and Macy, in press; Souders, Hunscher, Hummel and Macy, in press).

EXPERIMENTAL

The children selected were those whose medical records [2] showed a minimum of illnesses, none of which were extensive or severe, and the absence of known untoward hereditary factors in their histories; who were normal in weight for height by the standards of Meredith ('35) and Boynton ('36), and whose roentgenograms indicated skeletal development and maturity within normal limits.[3] Throughout the study the subjects received diets composed of twenty-one common foods [4] easily obtained throughout the year. The basal foods plus 400 gm. of milk yielded daily an average of 1676 calories (79.3 per kilogram of body weight); approximately 70 gm. of fat (3 per kilogram of body weight); 212 gm. of carbohydrate (9 per kilogram of body weight); 57.35 gm. protein, 5% of the fresh diet, and 17.8% of the dry weight (2.5 gm. per kilogram of body weight); 0.91 gm. calcium (0.041 per kilogram of body weight); 1.25 gm. phosphorus (0.056 per kilogram of body weight). Three young women, trained in nutrition and dietetics, quantitatively prepared and served the food and collected all the metabolic specimens which were analyzed [5] by the staff of the Research Laboratory of the Children's Fund of Michigan.

The milk used in the diets was pasteurized milk obtained from one creamery and subjected to identical processing and handling. During the initial study period, six children re-

[2] Marsh W. Poole, M.D., and Charles A. Wilson, M.D., attending physicians at the Methodist Children's Village, kindly made the medical examinations, the physical measurements and were responsible for maintaining the health of the children.

[3] The late T. Wingate Todd, M.D., and C. C. Francis, M.D., Western Reserve University, cooperated generously in a most helpful way by evaluating the skeletal maturity of all the children contacted, particularly those finally chosen for the metabolic study.

[4] The basal diet was composed of: apple, banana, beef, white and whole wheat bread, butter, cabbage, carrots, cheese, eggs, graham crackers, lettuce, milk, peanut butter, potato, shredded wheat or cornflakes, tomato and orange juice, sugar and salt.

[5] The chemical methods, omitted by space requirements, may be obtained from the authors. Methods of analysis will be presented in detail in a forthcoming monograph upon the chemical growth of children.

ceived 400 gm. of milk incorporated with their basal dietary daily for periods of 30 to 65 days (total of 225 experimental days); the other child received 600 gm. for 20 days. During the second period of observation, two of the children received 800 gm. of milk daily for 150 to 160 days (total of 310 experimental days) and the remaining five subjects ingested 1000 gm. daily for 20 to 40 days (total of 140 experimental days). One child was returned to an intake of 400 gm. daily for 20 days after receiving the 1000 gm. feeding daily. The protein, fat and acid-base values of a representative sample of the basal foods, and of 100 gm. of milk, are:

	Protein	*Calcium*	*Magnesium*	*Sodium*	*Potassium*	*Total base*
	gm.	*meq.*	*meq.*	*meq.*	*meq.*	*meq.*
Basal foods	46.8	12.4	16.6	73.2	42.5	144.7
100 gm. milk	3.1	5.9	1.0	2.1	3.8	12.8

	Fat	*Phosphorus*	*Chlorine*	*Sulfur*	*Total acid*	*Base minus acid* [1]
Basal foods	45.1	37.1	73.3	31.5	141.9	2.8
100 gm. milk	3.1	5.2	3.0	1.7	9.9	2.9

[1] The acid-base mineral value: milliequivalents of 'excess base.'

In the calculation of the acid-base value of food the fact that the organic forms of phosphorus and sulfur do not bind base is a source of error that is recognized but cannot be controlled. During growth, however, it is mitigated somewhat by the fact that part of the phosphorus and most of the sulfur are deposited in organic form in the soft tissues of the body. When the organically bound phosphorus and sulfur are metabolized they contribute acid to the excreta which is not represented as such in the food. Inasmuch as nothing is known of the proportion of the different forms of the several elements in the food it seemed best for all practical purposes, especially in this study of growing children, to consider all of the minerals as entering into the acid-base value and to use 2 as the valence of sulfur and 1.8 as the valence of phosphorus (Shohl, '23). This procedure is justified further on the basis of previous studies (Hummel, Hunscher and Macy, in press; Souders, Hunscher, Hummel and Macy, in press).

The basal diet used in the study, exclusive of milk, contained only 2.8 milliequivalents (28 cc. 0.1 N alkali) of base in excess of acid. The addition of milk to this diet enhanced its basic qualities and thereby caused considerable change in the relative proportions of the various elements and the extent of their contribution to the acid-base value of the food. The quantities of the different elements in the basal foods and the amounts of the same elements which are added by each increase of 100 gm. of milk, have been given in order to bring out more clearly the changes in the proportion of each constituent resulting from dietary change.

The largest difference between the acid-base values of the diets with and without 400 gm. of milk is attributable, for the most part, to the change in calcium content. There was an increase of nearly 200% in this element due to the milk, i.e., 400 gm. of milk contributed almost twice as much calcium to the intake as did all the basal food constituents; or, on the basis of the different levels of milk, each 100 gm. of milk supplied in the diet added 50% as much calcium as was supplied in all the other foods used in the diet. The inclusion of 400 gm. of milk raises the average daily excess base value of the diet from 2.8 to 14.4 milliequivalents, an increase of 11.6 milliequivalents; with 1000 gm. of milk the diet contained more than eleven times as great an excess of base over acid as the foods without any milk.

The next largest contribution of milk to the diet was phosphorus. The 400 gm. portion of milk contained slightly more than 50% of the amount of phosphorus found in all the other foods. When supplied in amounts of 800 gm. or larger it furnished the greater part of the phosphorus in the diet. In addition to calcium and phosphorus, the potassium and chlorine content of the diet was enhanced to a great degree, amounting to 89 and 42% increases, respectively, with the addition of 1000 gm. of milk. Milk is usually considered a poor source of sulfur, yet the sulfur content of the diet is augmented by 21, 31.7, 42.5 and 53% by the addition of 400, 600, 800 and 1000 gm. of milk, respectively. In a like manner

the protein in the total diet was increased daily from 59.1 to 77.5 gm. (26.3 to 65.8%), and the fat from 57.5 to 76.1 gm. (27.5 to 68.8%) when the milk levels were changed from 400 gm. to 1000 gm.

RESULTS

The average daily nitrogen and acid-base balances, together with the recumbent length and weight gains of the children, are shown in table 1. All of the subjects stored nitrogen, acid and base during the study. When the food intake was augmented by adding milk the retentions increased, as did the levels of excretion in the acid and base in both the urine and feces. The children with the highest intakes per kilogram of body weight, however, did not always have the highest retentions. D.P., the child with the lowest average daily intake per kilogram of body weight of both acid and base, 7.4 and 8.1 milliequivalents, respectively, showed the lowest average retention of base, 0.7 milliequivalent per kilogram daily, but not the lowest retention of acid. With 400 gm. of milk in the diet, the average excess of base over acid in the intakes was 0.6 milliequivalent per kilogram per day (6 cc. 0.1 N alkali) and the range, 0.5 to 0.7 meq./kg. When the level of milk ingestion was raised to 1000 gm. the average excess of base over acid in the intakes increased to 1.6 meq./kg./day. Four of the seven children showed a storage of excess base on all the levels of milk throughout the study. From the means and ranges of the intakes and retentions of total base, total acid, and excess base a marked correlation was observed.

Although the urine constitutes the major outlet of both acid and base, the urine of these subjects at all times contained an excess of acid over the base and the feces always contained more base than acid. Milk contributed appreciable quantities of fat to the diet, but the excretion of an excess of acid in the urine was practically unaffected by the dietary changes, while the amount of excess base in the feces (probably to a great extent in the form of calcium soaps) increased during ingestion of the larger quantities of milk (unpublished data). These

TABLE 1

Nitrogen and acid-base mineral balances of seven children during periods of ingesting diets differing in milk quantity

CHILD AND AGE	MILK INTAKE	LENGTH OF STUDY	RECUMBENT LENGTH		WEIGHT		NITROGEN	TOTAL BASE				TOTAL ACID				TOTAL BASE MINUS TOTAL ACID			
									Outgo				Outgo				Outgo		
			Initial	Daily gain	Initial	Daily gain	Retention	Intake	Urine	Feces	Retention	Intake	Urine	Feces	Retention	Intake	Urine	Feces	Retention
months	(gm.)	(days)	(cm.)		(kg.)		(mg./kg./day)	(meq. per kg. per day)				(meq. per kg. per day)				(meq. per kg. per day)			
D.P.:																			
99	400	65	135.4	0.034	27.79	0.002	2.1	8.1	5.5	1.9	0.7	7.4	5.9	1.0	0.5	0.7	—0.4	0.9	0.2
101	800	160	137.6	0.008	27.89	0.008	13.6	9.9	6.3	2.4	1.2	8.8	6.9	1.1	0.8	1.1	—0.6	1.3	0.4
W.P.:																			
96	400	30	119.4	0.003	22.88	0.010	7.8	9.5	6.2	2.5	0.8	8.8	7.2	1.2	0.4	0.7	—1.0	1.3	0.4
97	800	150	119.5	0.015	23.19	0.012	35.2	11.3	6.9	3.4	1.0	10.1	7.9	1.5	0.7	1.2	—1.0	1.9	0.3
C.D.:																			
123	600	20	134.2	0.015	33.95	0.028	43.8	9.7	5.0	2.5	2.2	9.2	5.8	1.2	2.2	0.5	—0.8	1.3	0.0
124	1000	20	134.5	0.020	34.50	0.030	60.7	12.4	5.4	3.1	3.9	11.1	6.2	1.4	3.5	1.3	—0.8	1.7	0.4
R.C.:																			
68	400	30	111.8	0.040	19.65	0.014	40.4	11.8	6.9	2.2	2.7	11.2	7.5	1.6	2.1	0.6	—0.6	0.6	0.6
70	1000	35	113.2	0.037	20.13	0.013	54.0	16.3	7.9	3.6	4.8	14.6	9.3	2.1	3.2	1.7	—1.4	1.5	1.6
J.L.:[1]																			
106	400	30	128.3	0.023	25.27	0.008	40.4	9.3	5.3	2.3	1.7	8.8	5.5	1.2	2.1	0.5	—0.2	1.1	—0.4
107	1000	25	129.0	0.025	25.50	0.006	60.2	13.0	6.3	3.3	3.4	11.6	6.9	1.8	2.9	1.4	—0.6	1.5	0.5
P.S.:																			
66	400	30	114.8	0.053	22.30	0.014	18.6	10.4	5.1	2.8	2.5	9.9	6.4	1.5	2.0	0.5	—1.3	1.3	0.5
67	1000	40	116.4	0.068	22.72	0.008	46.0	14.5	6.6	4.2	3.7	12.9	7.9	2.3	2.7	1.6	—1.3	1.9	1.0
C.L.:[1]																			
65	400	40	107.0	0.015	17.85	0.011	28.1	13.0	7.1	3.6	2.3	12.3	8.0	2.0	2.3	0.7	—0.9	1.6	0.0
66	1000	20	107.6	0.050	18.30	0.045	53.5	17.6	9.0	5.0	3.6	15.7	9.8	2.8	3.1	1.9	—0.8	2.2	0.5
67	400	20	108.6	0.080	19.20	0.000	35.2	12.2	6.6	3.0	2.6	11.6	7.5	1.9	2.2	0.6	—0.9	1.1	0.4
Means for 400 gm. intake (245 days)							21.1	10.3	6.0	2.6	1.7	9.7	6.7	1.5	1.5	0.6	—0.7	1.1	0.2
Means for 1000 gm. intake (140 days)							53.7	14.8	7.1	3.8	3.9	13.2	8.1	2.1	3.0	1.6	—1.0	1.7	0.9

[1] Female.

findings point to the importance of the bowel as a significant excretory pathway in the control of acid-base mineral balance, in spite of the effective mechanism used by the kidney in maintaining acid-base equilibrium of the body.

Table 1 illustrates differences among individuals as well as the changes in mean retentions corresponding to increases or decreases in intake. Considering both increase in intake and individual differences the Analysis of Variance Method (Snedecor, '34) enables us to conclude that the differences in mean retentions of total base, total acid and excess base are all statistically significant for the change from 400 to 1000 gm. of milk; when the change in level of milk intake was from 400 to 800 gm. per day the differences in the mean retentions of total base and total acid were statistically significant. Although the changes in quantity of mineral elements ingested produced corresponding and significant differences in the retentions, it is apparent that the retentions of the different children are primarily characteristic of their inherent physiologic needs.

The children showed gains in recumbent length and weight during the course of the study, but the amounts of these gains seem to have no direct relationship with the quantity of excess base stored. The children all retained nitrogen throughout the study, ranging from 2.1 to 40.4 mg. per kilogram of body weight daily on the 400 gm. level of milk intake to 46.0 to 60.7 mg. during the 1000 gm. intake. The lack of relationship between the amount of storage of excess base and gains in weight or recumbent length is best illustrated by C.L. This child received 400 gm. of milk in her diet for a period of 40 days, during which time she maintained acid-base mineral equilibrium and gained in recumbent length and weight, 0.015 cm. and 0.011 kg. per day respectively. During this period she stored, on the average, 14.9 mg. calcium and 28.1 mg. of nitrogen per kilogram of body weight. For the next 20 days 1000 gm. of milk were ingested daily; the nitrogen and calcium storages rose to 53.5 and 28.9 mg. per kilogram of body weight respectively, and the retention of excess base increased to

0.5 milliequivalent while the gain in recumbent length rose to 0.05 cm. and the weight to 0.045 kg. daily. During the last 20 days of the experiment this child was returned to a 400 gm. level of milk. The storage of excess base dropped only slightly to 0.4 milliequivalent, the nitrogen to 35.2 mg. and the calcium to 15.2 mg. per kilogram daily. The recumbent length rate of gain, however, continued to increase to 0.08 cm. daily, while the weight remained stationary.

These results indicate that neither the recumbent length, the weight gains, nor the excess base balance, alone, give an index to the type of chemical growth that is taking place. The retention of nitrogen is a more exact measure of growth than is the increase in weight, which may be due in a varying degree to body water or fat. Space does not permit discussion of the interrelation of the various acid-base mineral elements in growth, but this will be the subject of future publications. Because of the accuracy and frequency with which the recumbent length measurements and weights were taken, however, they contribute significant information which correlates with the metabolic balances of eleven different chemical elements and constituents in specific types of chemical interrelationships. With the changes to higher levels of milk intake the increased calcium: phosphorus ratios indicate gains in rate of synthesis of skeletal tissue over the rate of soft tissue construction, and bear out the fact that the kind of chemical growth accruing at any particular time is characteristic of the individual child with his peculiar inherent physiologic needs in response to the growth impulse as well as to the type and quantity of food that he consumes.

It seems apparent from these data that the extra base supplied by the additional quantities of milk caused a greater positive base balance in these seven growing children, although unrelated quantitatively to the actual gains as measured by recumbent length and weight. The children were at all times storing both basic and acidic minerals, even on the mixed diet with 400 gm. of milk, but the base balance was not always positive during the lower level of milk ingestion. It was not

until 1000 gm. of milk were given daily that the level of excess base retention for these children approximated the 10 cc. of 0.1 N base suggested by Shohl ('23) as being desirable for growth.

SUMMARY

The nitrogen, acid (phosphorus, chlorine, sulfur), base (calcium, magnesium, sodium, potassium) and 'excess base' balances are given for seven healthy children, ages 5 to 10 years, over 715 experimental days, when they were consuming 400, 600, 800 and 1000 gm. of milk daily. Not only are the various quantities of the elements changed in the diet but the proportion of one to the other is appreciably changed when different levels of milk are incorporated in a known fixed dietary.

Due to the inherent physiologic conditions characteristic of the individual child and not always amenable to control or adjustment, each child served as his own control and was observed on two different levels of milk. Again, due to the variability of metabolism from day to day, especially in children, each observation period extended over 20 or more consecutive days on a given intake of milk, but in one case it extended as long as 160 consecutive days. The basal diet was kept constant in quality of mixed foods though the quantity was adjusted to meet the physiologic needs of the individual child; the average daily intake including 400 gm. of milk per kilogram of body weight amounted to: 79.3 calories, 3 gm. of fat, 9 gm. of carbohydrate, 2.5 gm. of protein, 41 mg. of calcium and 56 mg. of phosphorus.

All children were storing both acid and base throughout the entire study but the quantity stored was greater during ingestion of the larger amounts of milk. The mean daily balance of 'excess base' for all the children on the 400 gm. intake of milk was 0.2 meq. (2 cc. 0.1 N base) per kilogram of body weight as contrasted with 0.9 meq. (9 cc. 0.1 N base) during the daily ingestion of 1000 gm. of milk.

LITERATURE CITED

BOYNTON, B. 1936 The physical growth of girls. A study of the rhythm of physical growth from anthropometric measurements on girls between birth and eighteen years. Univ. of Iowa Studies, Studies in Child Welfare, vol. 12, no. 4.

COOLEY, T. B., G. C. PENBERTHY, L. ARMSTRONG, H. A. HUNSCHER, F. COPE AND I. G. MACY 1935 Mineral metabolism. A case of osteopsathyrosis and one of ununited fracture. Am. J. Dis. Child., vol. 50, p. 431.

GREENWALD, I. 1922 A normal diet. Endocrinology and Metabolism, ed. by L. F. Barker, New York and London, vol. 3, p. 413.

HUMMEL, F. C., H. A. HUNSCHER AND I. G. MACY 1939 The influence of fluid irradiated and evaporated irradiated milks on the storage of nitrogen and acid-base minerals in children. Am. J. Dis. Child. (In press.)

MEREDITH, H. V. 1935 The rhythm of physical growth. A study of eighteen anthropometric measurements on Iowa City white males ranging in age between birth and eighteen years. Univ. of Iowa Studies, Studies in Child Welfare, vol. 11, no. 3.

SAWYER, L., L. BAUMANN AND F. STEVENS 1918 Studies of acid production. II. The mineral loss during acidosis. J. Biol Chem., vol. 33, p. 103.

SHOHL, A. T. 1923 Mineral metabolism in relation to acid-base equilibrium. Physiol. Rev., vol. 3, pp. 509.

SNEDECOR, G. W. 1934 Calculation and interpretation of analysis of variance and covariance. Collegiate Press, Ames, Iowa.

SOUDERS, H., H. A. HUNSCHER, F. C. HUMMEL AND I. G. MACY 1939 A comparison of the influence of fluid and evaporated milks on mineral and nitrogen metabolism of growing children. Am. J. Dis. Child. (In press.)

STEARNS, G. 1931 The significance of the retention ratio of calcium: phosphorus in infants and children. Am. J. Dis. Child., vol. 42, p. 749.

WHITE HOUSE CONFERENCE ON CHILD HEALTH AND PROTECTION 1932 Growth and development of the child. Part III. Nutrition. The Century Co., New York and London, p. 213.

THE INFLUENCE OF EXERCISE ON THE GROWING RAT IN THE PRESENCE AND ABSENCE OF VITAMIN A[1,2]

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FIVE FIGURES

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Research literature in the field of nutrition abounds in unexplained variations and discrepancies owing to numerous biological variables which are difficult if not impossible to control. This is especially true of those data obtained in investigations dealing with various phases and forms of malnutrition. Investigators have long recognized certain influencing factors and have attempted to determine the relative magnitude of their respective influences on the ultimate experimental results. The importance of heredity and previous nutritional history of the experimental subjects, together with such factors as age, sex and size, have been rather extensively investigated. Other factors including radiant energy and environmental temperatures and humidities have also received consideration. Although it has been frequently assumed that a relationship exists between the physical activity of an experimental animal and its nutritional response, little actual experimental work has been done toward establishing the relative significance of this factor, and these efforts have been directed mainly from the standpoint of energy requirements.

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[2] Presented before the division of biological chemistry at the Milwaukee meeting of the American Chemical Society, September 5 to 9, 1938.

It appears that adequate investigations have not been conducted regarding either the relationship of physical activity to the vitamin requirement of various species of animals or to the effect of vitamin intake on the inclination of such animals to exercise. Yoshiue ('22) reported that vitamin B deficient rats which were exercised died before developing the muscular paralysis typical of this deficiency. Blacklock ('24), on the other hand, has expressed a belief that lack of exercise accelerates and accentuates the ill effects of diets deficient in vitamin B. This author cites the fact that the disease beri beri most frequently occurs in institutions and in those situations where a fixed diet and some degree of confinement are usually maintained. Keith and Mitchell ('23) placed rats receiving a vitamin A deficient diet in cylindrical boxes and rotated the boxes at the rate of thirteen revolutions per minute for periods ranging from 30 to 40 hours per week. As a result of the studies these authors concluded that exercise distinctly hastened the appearance of the symptoms of vitamin A deficiency and hastened the death of young animals receiving vitamin A deficient diets. Similar studies carried out by these authors while employing diets deficient in vitamin B did not show that the requirement for this dietary factor was affected by exercise. In addition to these citations, numerous authors have inferred that they recognized certain influences of exercise on vitamin requirement as well as increased physical activity as the result of adequate vitamin intake, although no data were given in support of such statements.

It would seem that if exercise does influence vitamin requirement some of the variations in individual response of experimental animals comprising a carefully selected group might be due to differences in physical activity of different animals in the group, and also that differences in responses obtained by different investigators might be at least partially explained on the basis of the difference in the degree of freedom allowed the experimental subjects. With these thoughts in mind it appeared that the influence of exercise or activity of the experimental animal on its vitamin requirements and

also the effect of vitamin intake on physical activity merited further study. The present report is concerned primarily with the influence of exercise on the development and well being of the growing rat in the presence and absence of vitamin A. Similar studies are now under way with reference to other vitamins.

EXPERIMENTAL

The investigation consisted essentially of a comparison of the physiological activity and growth responses of comparable groups of young rats while being maintained under identical conditions with the exception of the amount of exercise permitted by the types of caging employed and of the vitamin A intake. The experimental equipment consisted of three sets of individual all-metal cages, of which two sets were of rotating type, having a wheel approximately 14 inches in diameter that was equipped with a registering cyclometer. One set of the rotating cages was so arranged on one side of a large table that the wheels could be rotated at a definite speed by means of a continuous belt driven by an electric motor through a gear reducing mechanism. The second set of rotating cages was arranged on the opposite side of the table and the wheel of each cage was adjusted so as to allow uniform freedom of rotation. The third set of cages consisted of our regular laboratory cages, each of which had approximately 120 square inches of floor space. All cages were provided with raised screen floors. Clean paper was kept under the cages.

Healthy rats, 21 to 23 days of age and weighing approximately 45 gm., were used as the experimental subjects. A special effort was made to place comparable animals of the same litter, sex and body weight in the three respective groups. All animals were fed ad libitum a vitamin A deficient basal diet which consisted of: purified casein 18, salt mixture 3, Cell-U-flour 2, dextrinized cornstarch 77 and irradiated yeast 8. Clean distilled water was available at all times.

Each set of animals was subdivided into two groups. Animals of one group received only the vitamin A deficient diet until depleted of their body reserve of this vitamin, while the animals of the second group served as positive controls and in consequence each received 2 U.S.P. units of vitamin A daily. After being depleted, the animals of the former group also received this quantity of vitamin A daily for a period of 35 days.

The animals of the forced exercise group were allowed access to the rotating wheel only during the period of rotation. During the remainder of the time they were confined in the small side cage (60 square inches of floor space) containing the food and water. The animals of the voluntary exercise group and those housed in the stationary cages were allowed free access to the entire cage at all times.

Observations made on a group of normal young rats which received our breeding colony diet, housed in the above type of rotating cage and allowed freedom of exercise, had shown that an average of 1000 revolutions per day was made during a period of 70 days. This number of revolutions was taken, therefore, as the basis for the maximum daily rotation of the forced exercised groups. Previous observations had also indicated that a speed of approximately six revolutions per minute, with this particular type of cage, was the most effective in keeping the animals in constant motion. Speeds less than six revolutions per minute allowed the animals to stop frequently, while greater speeds tended to cause the animals to cling to the wheel and 'ride' instead of exercising as was desired. At the above rate of rotation, approximately 3 hours per day were required to rotate the cages of the forced exercise group 1000 revolutions or a distance of approximately 0.7 mile. This period of rotation was always started in the early forenoon following the weighing of the animals. At the end of the rotation period each animal was again returned to the small side cage containing the food and water where it remained until the following day.

A daily record was made of the weight of the animal, the amount of food consumed, the number of fecal pellets and the number of revolutions made by the wheels of the rotating cages during the previous 24 hours. All fecal matter was removed from the cage pans daily and stored in glass containers. At the end of each week the fecal collection from each rat was dried and weighed. Daily observations were also made of the animals in an effort to detect the onset as well as the relative stage of the symptoms characteristic of vitamin A deficiency. As the body weight of each animal ceased to increase and symptoms of xerophthalmia appeared in one or both eyes, the animal was given daily doses of 2 U.S.P. units of vitamin A as a supplement to the deficient diet (see above). The duration of the experimental periods for all animals ranged from 64 to 71 days, depending upon the time required to deplete the respective animals. Those animals receiving the vitamin A supplement from the beginning of the experiment were continued under observation for a period of 70 days.

DATA

In order to conserve space the data obtained for individual animals have been reduced to group averages and are presented in the following charts (see figs. 1 to 5 inclusive). Only data from those animals which survived the entire experimental period have been used in tabulating these summaries.

DISCUSSION

It was observed at the beginning of the experiment that those animals housed in the rotating cages and composing the forced exercise group were unable to undergo the full 1000 revolutions before becoming completely exhausted. In order to avoid any possible vitiating effect of over exhaustion on subsequent responses, these animals were exercised during the first few days of the experiment to the point of partial exhaustion and the number of revolutions was increased daily until the end of the first week at which time the cages were undergoing the desired 1000 revolutions daily.

It may be observed from the data presented in figure 1 that exercise, under the conditions of this experiment, had a measurable depressing effect on the rate of growth of young rats irrespective of the vitamin A intake. The effect of this factor on the rate of growth was, however, somewhat greater

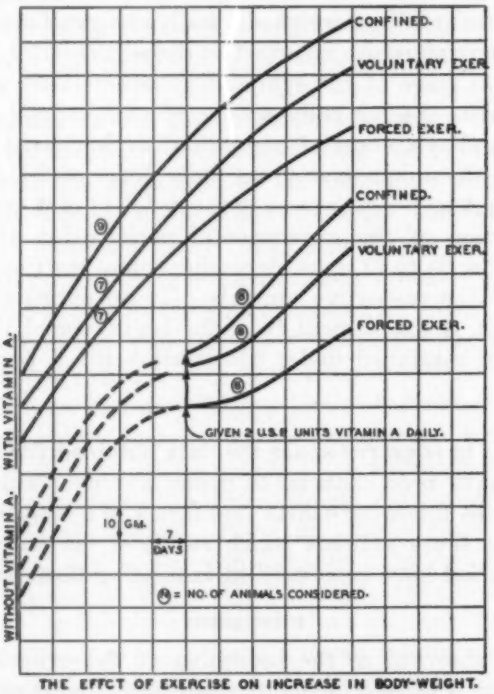

Fig. 1 The effect of different amounts of exercise on the growth rate of young rats while being maintained under comparable dietary conditions except as to their vitamin A intake.

among those animals which had been previously depleted of their body store of vitamin A. These findings are further emphasized by the data presented in figure 2 relative to the effect of exercise on the total change in body weight during the entire experimental period. Here again the animals of

the confined groups, both with and without vitamin A, made the greatest increases in body weight. No attempt was made to determine the chemical nature of the weight increases of the respective groups of animals.

The data presented in figure 2 concerning the total food intake of the various groups of animals are somewhat surprising in that it was expected that greater physical activity would result in greater food consumption in order to compensate for the greater energy exchange involved. Such is

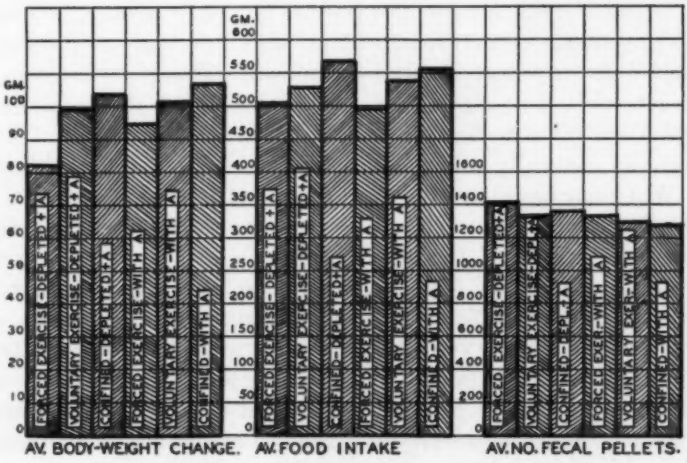

Fig. 2 The relative effect of exercise on the average body weight increase, average food intake and average number of fecal particles voided by young rats receiving a diet with and without vitamin A.

not indicated by the data obtained, for in each instance the group of animals undergoing the least amount of exercise consumed the greatest amount of food. Similar results have been reported by Keith and Mitchell ('23). While no explanation can be offered at this time for the suppressing effect of exercise on food intake, it seems highly probable that the physical effects of the 3 hours of rotation during the forenoon are important considerations.

Another unexpected observation is the close similarity in the total amounts of food consumed by the animals of the corresponding groups irrespective of their vitamin A intake. These findings can at least be partially explained on the basis of the high food intake of the depleted animals during the 35 days the vitamin A supplement was fed.

Figure 2 also contains data concerning the effect of exercise on the average number of fecal pellets voided by the different groups of animals during the entire experimental period. It will be observed that while the number of fecal pellets voided by the different groups of rats did not vary widely, in each instance those animals composing the forced exercised groups voided the greatest number of pellets. These findings may be interpreted as signifying a favorable effect of exercise on intestinal motility and, in consequence, on elimination. Exercise is generally believed to have a similar effect on humans.

When one considers the total weight of the fecal matter, the relative proportions of food excreted as fecal matter and the average weight of the fecal pellets voided by the various groups of animals (fig. 3) differences between the groups become more significant. In all instances those animals of the confined groups voided the greatest weight of fecal matter, the greatest percentage of fecal matter and the heaviest fecal pellets. The lower percentage of fecal matter from the animals of the exercised groups might be explained on the basis that these animals were more efficient in the utilization of part or all of the constituents of their food than were comparable groups of animals which were not exercised. The smaller fecal pellets from the exercised animals further indicate increased intestinal motility among the animals of these groups.

From the data presented in figure 4 it may be observed that exercise has a significant effect on the time required for the cessation of growth and for the onset of xerophthalmia among rats receiving a diet deficient in vitamin A. The values given in these graphs represent the averages for all animals of the respective groups. It may be seen that it required, on

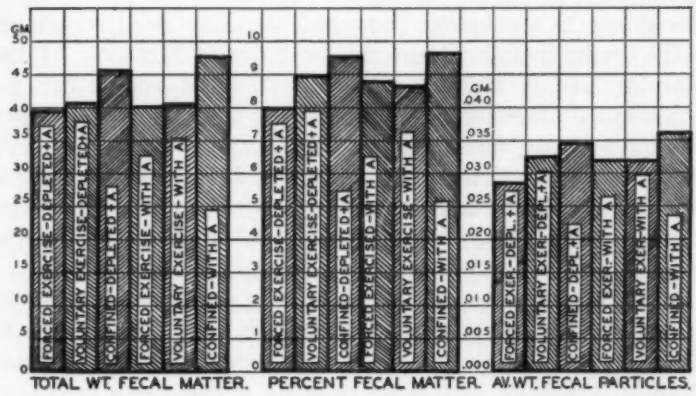

Fig. 3 The effect of exercise on the total weight of fecal matter, percentage of fecal matter and average weight of fecal particles voided by young rats while receiving diets comparable in all respects except as to the vitamin A content.

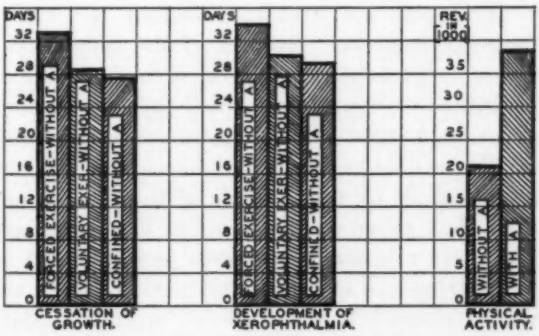

Fig. 4 The effect of exercise on the average time required for the cessation of growth and for the development of the symptoms of xerophthalmia among young rats receiving a diet deficient in vitamin A. This figure also shows the relative activity (as measured by rotation of the cage wheel) of comparable groups of young rats, of which the animals of one group received daily 2 U.S.P. units of vitamin A throughout the experiment, while the animals of the other group received no vitamin A supplement during the first part of the experiment but received 2 U.S.P. units of vitamin A daily during the last 35 days.

an average, 33 days to bring the animals of the forced exercise group to a constant body weight, while similar animals in the confined cages required an average of 27.5 days. Likewise, an average of 34 days was required to produce definite symptoms of xerophthalmia among the animals of the forced exercised group, while those of the confined group required only 29.6 days. These data are not in agreement with those obtained by Keith and Mitchell ('23). However, the two experimental procedures can hardly be considered comparable. It seems possible that the vitamin A requirement of a young animal may be more definitely related to its rate of growth than to its physical activity. It has been frequently observed in this laboratory in connection with our regular vitamin A assays that those animals which make the most rapid rate of growth during the depletion period also are the first to develop the symptoms of vitamin A deficiency. Hence, it appears logical to assume that the confined animals which made the greatest increase in body weight and in consequence developed the greatest demand for the vitamin, should show the earliest symptoms of the deficiency.

Data are also presented in figure 4 to show the effect of vitamin A intake on the physical activity of two comparable groups of animals while being maintained in the freely rotating cages. These data represent the average number of voluntary rotations made by the animals of the two groups. It is to be noted that those animals which had been previously depleted of their vitamin A reserves made a total of 22,000 revolutions during the experimental period, while those animals which received 2 U.S.P. units of vitamin A daily made a total of 38,000 revolutions in a similar period.

It was also observed that a number of the animals composing the voluntary exercise group did not register an appreciable number of revolutions during their first week in the rotating cages. But with increased familiarity with their cage-quarters, all animals of this group showed greater inclination to rotate the wheel. In the case of those animals receiving 2 U.S.P. units of vitamin A daily (fig. 5), this in-

crease in activity continued throughout the experimental period but did not equal the activity previously observed among young animals receiving the breeding colony diet. Those animals which received the vitamin A deficient diet without supplement showed weekly increases in the number of rotations until the end of the fourth week when slight evidences of vitamin A deficiency were becoming apparent. During the following week, these animals showed a marked decrease in activity which continued through the seventh week,

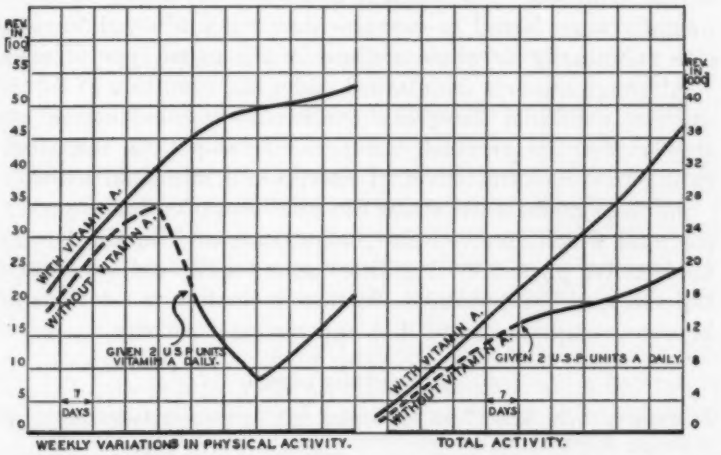

Fig. 5 The average weekly variation in physical activity and also the total activity of two groups of young rats maintained under comparable conditions in all respects except as to their vitamin A intake.

although each animal of this group received 2 U.S.P. units of vitamin A daily beginning with the sixth week of the experiment. During the eighth, ninth and tenth week, all animals of this group showed a definite return to activity. In spite of this rapid return to activity the average number of rotations made by this group of animals was always considerably less than the number of rotations made during corresponding weeks of the experiment by the animals in a comparable group which had received the vitamin supplement

from the beginning of the experiment. These findings further emphasize the importance of an adequate vitamin A intake in the promotion of vigorous physical activity.

SUMMARY

Experiments carried out with carefully selected groups of rats as experimental subjects yielded data to show that under comparable conditions of experimentation less food was consumed, smaller increases in body weight were made and less severe symptoms of vitamin A deficiency developed when the animals were forced to exercise than when allowed to exercise voluntarily or when confined in the usual type of cage.

Although animals maintained under the condition of forced exercise exhibited the greatest efficiency of food utilization, they voided the greatest number of fecal pellets, thus indicating the beneficial effect of exercise on intestinal motility.

Animals maintained under the condition of voluntary exercise and which received daily allotments of vitamin A were more active physically than litter mates which did not receive the vitamin A supplement, further indicating a relationship between completeness of diet and physical activity.

LITERATURE CITED

BLACKLOCK, D. B. 1924 Lack of exercise as a determining factor in the epidemiology of beri beri. Brit. Med. J., no. 3311, pp. 1046–1047.

KEITH, M. HELEN, AND H. H. MITCHELL 1923 The effect of exercise on vitamin requirement. Am. J. Physiol., vol. 65, pp. 128–138.

YOSHIUE, S. 1922 Über den Einfluss der Funktion auf den Verlauf der Avitaminosen. Biochem. Z., Bd. 134, S. 375–380.

THE RELATION OF DIETARY FAT TO THE THIAMIN REQUIREMENTS OF GROWING RATS [1, 2]

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THREE FIGURES

(Received for publication December 14, 1938)

There still appears to be some question as to the effect of increased amounts of fat in the diet on the thiamin requirements of animals. As Evans, Lepkovsky and Murphy ('34) have pointed out, Funk ('14) early suggested that the severity of thiamin deficiency symptoms could be correlated with the amount of carbohydrate in the diet. There is now ample evidence that thiamin is related to the metabolism of pyruvic acid.

The first report to show definitely that rats can be maintained with greatly reduced thiamin intakes when the ration contains high levels of fat was that of Evans and Lepkovsky ('28). Salmon and Goodman ('37) have studied the question recently and a review of the literature is included in their paper.

We became interested in this problem for two reasons. First, we had available a basal ration which was complete in all nutrients except thiamin. This ration, described by Arnold and Elvehjem ('38), gives normal growth in rats when supplemented with adequate thiamin. Second, we felt that much

[1] Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

[2] Presented in part at the thirty-second annual meeting of the American Society of Biological Chemists, March, 1938 (Stirn and Arnold, '38).

[3] Now with U. S. Standard Products, Woodworth.

[4] Wilson and Company fellow.

more uniform results would be obtained if the sucrose in the basal ration was replaced by the fat on an isocaloric basis. This procedure would not alter the ratio of the remaining constituents of the diet.

EXPERIMENTAL

Composition of rations. Table 1 gives the composition of basal ration 112 as well as the percentage composition of the modifications containing varying amounts of fat. In these modifications one-fourth (ration 112a), one-half (ration 112b),

TABLE 1

Composition of diets showing procedure in replacing sucrose by isocaloric amounts of fat

CONSTITUENTS	R. 112		R. 112a		R. 112b		R. 112c		R. 112d	
	parts	%	*parts*	%	*parts*	%	*parts*	%	*parts*	%
Sucrose	60	60	45	49.2	30	36.2	15	20.1		
Fat			6.5	7.1	13	15.7	19.7	26.2	26	39.4
Purified casein	18	18	18	19.7	18	21.7	18	24.1	18	27.3
Factor W = liver extract	2	2	2	2.2	2	2.4	2	2.7	2	3
Autocl. peanuts	10	10	10	10.9	10	12.1	10	13.5	10	15.2
Autocl. yeast	4	4	4	4.4	4	4.8	4	5.4	4	6.1
Salts I	4	4	4	4.4	4	4.8	4	5.4	4	6.1
Paper pulp	2	2	2	2.2	2	2.4	2	2.7	2	3
Total	100[1]	100	91.5	100	83	100	74.7	100	66	100
Caloric ratio sucrose/fat	100% sucrose		75/25		50/50		25/75		100% fat	

[1] Vitamin A and D supplied separately to all animals as a concentrate.

three-fourths (ration 112c) and all of the sucrose (ration 112d) have been isocalorically replaced by fat. It must be mentioned that the autoclaved yeast and the autoclaved peanuts supply about 6.5% fat to ration 112. The figure for the amount of fat in the modifications of ration 112 does not include the fat supplied by these ingredients.

The animals. Albino rats were placed on experiment at about 25 days of age at a weight of 40 to 50 gm. The animals were kept in individual cages equipped with wire screen bottoms (two meshes to the inch). Distilled water was ob-

tained from inverted bottles suspended on the side of each cage. Growth records were obtained from weekly weighings.

Procedure. The rats were depleted of their thiamin reserves by restriction to ration 112 until the symptoms of polyneuritis appeared. Occasionally the rats had limited their food intake to the extent that cures were not obtained when rations high in fat were offered to the rats. The number of such animals was very small and with more careful observation such animals were rarely encountered.

Comparison of growth of rats fed ration 112 supplemented with thiamin chloride and ration 112d without added vitamin. The composite growth curve of three rats (male) which had been polyneuritic and which had then been fed thiamin

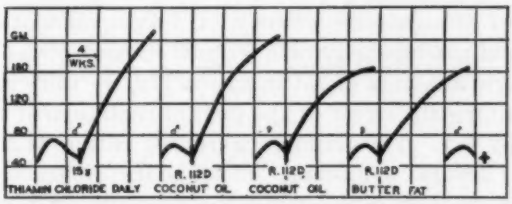

Fig. 1 Growth records of rats fed ration 112 to deplete their thiamin reserves and then fed either ration 112 supplemented with 15 micrograms of thiamin chloride daily or ration 112d using either coconut oil or butterfat to isocalorically replace the sucrose component of the ration.

chloride are shown in figure 1. The amount of thiamin chloride fed (15 micrograms daily) insured good growth. Similar growth resulted with the three animals (male) which had been brought out of the polyneuritic condition by the isocaloric substitution of fat for carbohydrate (ration 112d) in the diet. In this instance coconut oil was used. The animals which received the vitamin supplemented ration 112 gained 20.1 gm. per week for the following 6 weeks. The animals fed ration 112d (coconut oil) gained 20.5 gm. per week for the comparable period. Three rats (female), grew, as would be expected, at a somewhat slower rate (fig. 1). The average for the three was 14.5 gm. per week for the 6-week period follow-

ing the change in rations. The use of butterfat rather than coconut oil as in the previous groups made no appreciable difference in the growth rates of five rats (female) (fig. 1). The curve shown in figure 1 shows a gain of 14.0 gm. per week for the 6-week period following the change in diets. The individual growth records of the rats are not significantly different from the composite growth records as given in the figure. The three control animals fed ration 112 died at the end of the fourth week on the diet.

These results satisfied us that a vitamin sparing action could easily be demonstrated on a diet which was adequate in all the nutritive factors needed by the rat aside from thiamin.

Results of partial replacement of the carbohydrate by fat. Because of the extreme efficacy of fat in alleviating polyneuritis and producing growth when the sucrose was replaced by isocaloric amounts of fat in ration 112, we were interested in determining the effect of the partial replacement of carbohydrate by fat. The animals were fed ration 112 until all exhibited severe symptoms of thiamin deficiency. The animals were then divided into four groups and placed on ration 112a, 112b, 112c and 112d respectively in which coconut oil partially or completely replaced the sucrose.

The growth of the animals in this experiment is shown in figure 2. Ration 112a, which contained 7.1% coconut oil, was able to alleviate the polyneuritic symptoms. The recovery period, however, was much prolonged. Growth was poor and amounted to only 5.0 gm. per week for the 7-week experimental period. Ration 112b, which contained 15.7% fat, was more effective in curing the symptoms and allowed an average growth of 5.3 gm. per week. Ration 112c, which contained 26.2% fat, produced results similar to those obtained on ration 112d. The former ration allowed growth of 10.3 gm. per week for the 7-week period while the animals fed the latter ration gained 15.4 gm. per week for the same period.

The animals fed ration 112d, which contained cottonseed oil, coconut oil or olive oil (fig. 2), were continued for 27 weeks

on the high fat rations. The female animals in these groups had normal estrous cycles as exhibited by vaginal smears. At the close of the experiment the females weighed 180 to 200 gm. and the males about 220 to 260 gm. The animals were apparently normal in every respect.

The effect of various levels of synthetic tricaproin. The results obtained with synthetic tricaproin are most interesting. The polyneuritic symptoms slowly disappeared when the animal was shifted to ration 112a (7.1% synthetic tricaproin).

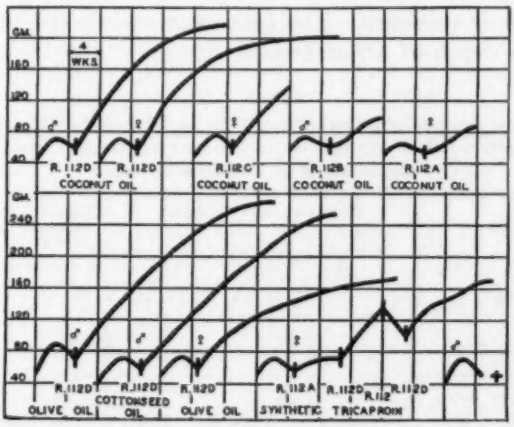

Fig. 2 Growth records of individual rats fed ration 112 to deplete their thiamin reserves and then fed coconut oil at levels which isocalorically replaced one-fourth (ration 112a), one-half (ration 112b), three-fourths (ration 112c) or all (ration 112d) of the sucrose in the diet. Also growth records of individual rats depleted as before and then fed ration 112d where olive oil, cottonseed oil or synthetic tricaproin (two levels) isocalorically replaced the sucrose.

Growth, however, was poor (2 gm. per week) for the 6-week period. This animal was then fed ration 112d (39.4% tricaproin) and immediately gained in weight. During the 5-week period the gain was 10.2 gm. per week. Ration 112 was then substituted for 3 weeks during which time there was a loss in weight of 4.5 gm. per week. Although this animal had been on experiment for 19 weeks, when again fed ration 112d

(tricaproin) it made an average gain of 7 gm. per week for the following 10 weeks.

This demonstrated that the requirement for thiamin was markedly reduced by as little as 7.1% synthetic tricaproin. It further showed that an animal could be made to gain or lose in weight merely by varying the amount of fat and sucrose in the thiamin deficient ration.

The effect of autoclaving on the thiamin sparing action. A study was made to determine whether the activity of natural fats would be decreased by autoclaving the fats for 10 hours at 15 pounds pressure. The fats were autoclaved in shallow pans at a depth of about ¼ inch.

TABLE 2

Weekly growth response of polyneuritic rats during the first 3 weeks on ration 112d (autoclaved coconut oil) and on ration 112d (autoclaved coconut oil) supplemented with thiamin chloride

RATION	AVERAGE GAIN IN WEIGHT			APPROXIMATE TIME FOR RECOVERY FROM POLYNEURITIC SYMPTOMS
	1st week	2nd week	3rd week	
	gm.	gm.	gm.	
Ration 112d (six rats)	23.5	17.7	16.5	3 days
Ration 112d + 15 micrograms thiamin chloride per day (six rats)	30.6	20.0	17.3	5 hours

The results in table 2 show the growth response of polyneuritic animals cured on ration 112d (autoclaved coconut oil) as compared with autoclaved coconut oil supplemented with 15 micrograms of thiamin chloride per animal per day. It was quite evident that the vitamin gave a supplementary effect only during the first week. This effect gradually decreased until at about the sixth week both groups made equal gains. Animals which received the vitamin supplement were apparently free from polyneuritic symptoms within 4 to 6 hours after the administration of the vitamin and immediately began to consume the fat ration. Animals on the unsupplemented fat rations regained their appetites more slowly.

The above animals were sacrificed at the end of the third week for determinations of the cocarboxylase content of the livers and brains.[5] The tissues of rats fed the high fat diets were compared with similar tissues from polyneuritic animals and animals which were fed the stock colony ration. The

TABLE 3

Cocarboxylase content of the livers and brains of rats fed ration 112, ration 112d or the colony stock ration

RATION	COCARBOXYLASE CONTENT	
	Liver	Brain
	cmm. CO_2	*cmm. CO_2*
Colony stock ration	536	381
Ration 112 (rats polyneuritic)	187	170
Ration 112d	205	318

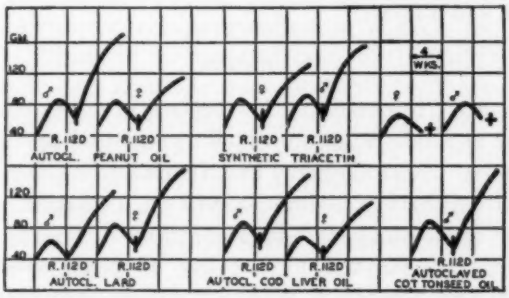

Fig. 3 The growth records of individual rats fed ration 112 until polyneuritic symptoms appeared and then fed ration 112d in which synthetic triacetin, autoclaved peanut oil, lard or cod liver oil isocalorically replaced the sucrose component of the ration.

results are summarized in table 3. It is evident that the livers from the rats fed the high fat rations are very similar to the tissues from polyneuritic rats, while those getting the normal ration are definitely higher. The brains do not show such great differences.

[5] The cocarboxylase values were kindly determined by Mr. M. A. Lipton according to the procedure of Lipton, Potter and Elvehjem ('38).

Differences with various diets. The response of polyneuritic animals to ration 112d using various other autoclaved natural fats which included peanut oil, lard, cod liver oil and cottonseed oil is shown in figure 3. Synthetic triacetin was also tested. All fats effectively alleviated the polyneuritic symptoms and supported growth. A limited number of animals in some groups which had gained weight the first week still showed mild polyneuritic symptoms. These symptoms usually disappeared within 8 days. Of this group the three females on peanut oil, lard and cod liver oil, and the male on cottonseed oil showed severe symptoms at the end of the first week yet the growth response was 10, 22, 8 and 13 gm. respectively. The fact that growth resulted in the presence of polyneuritic symptoms indicated that fats produced their effect independently of thiamin since the vitamin cured polyneuritis in 4 to 6 hours.

DISCUSSION

The results presented in this report are, we believe, not entirely at variance with the results presented by the earlier investigators. Since Gregory and Drummond ('32) were not able to control their experiments with pure thiamin, it is most likely that these workers had a multiple deficiency in their diets which was masked by the use of yeast concentrates. Kemmerer and Steenbock ('33) concluded that fats had no vitamin sparing action since the livers and muscle tissues of the rats fed the high fat diets contained no more thiamin than did the rats fed the vitamin low basal ration. The data presented in table 3 corroborate these findings. The livers of the rats fed the high fat diets are significantly lower in cocarboxylase than the livers from rats fed the colony stock ration. The evidence, therefore, shows that while the animals are unable to build up or maintain their thiamin reserves they are still able to obtain energy from the metabolism of fat for growth purposes. The data in table 3 add further evidence for the view that the basal ration is extremely low

in its thiamin content although we are unable completely to rule out the possibility that it does carry traces of the vitamin.

In certain respects the results presented here do not agree with those of Salmon and Goodman ('37). These investigators observed 100% incidence of polyneuritis in rats fed a thiamin low ration which contained 40% of cottonseed oil, pecan oil, linseed oil, soft lard, beef fat, olive oil or butterfat. The average time for the onset of the polyneuritis symptoms in the rats fed the latter three fats was apparently the same as that on the low fat ration (p. 484, table 3, 37 days). The nutritive ratio of the 40% fat diet used by these workers was approximately 5.7 : 1 which corresponds to a low fat diet with about 15% protein. It is possible that the diets fed by these investigators were low in factors other than thiamin since growth responses also resulted when autoclaved yeast was added to the diet (p. 483, fig. 1). It may be noted that when the autoclaved yeast supplemented to a high fat diet was increased sufficiently no cases of polyneuritis were observed (p. 484, table 3). It is also seen that even in the presence of adequate thiamin (p. 483, fig. 2, curve 5) growth was less than 2 gm. per day during the first 8 weeks on experiment. We have previously found (Arnold and Elvehjem, '38) that even autoclaved yeast cannot always be depended upon to supply adequate amounts of the members of the vitamin B complex other than thiamin.

Whipple and Church ('36) have suggested that thiamin is necessary for fat synthesis because animals on a thiamin low diet have a decreased fat reserve while the reserve is increased upon the addition of the vitamin. The same conclusions were arrived at as a result of studies on the respiratory quotient (Whipple and Church, '37). It is not possible from any present work to determine whether thiamin is specifically necessary for fat synthesis. However, we would like to point out that the decreased fat stores can be explained in a slightly different way in the light of our results. In thiamin deficiency the rat preferentially metabolizes fat as opposed to carbohydrate. This wipes out the fat reserves.

In the presence of thiamin the rat is able to metabolize carbohydrate normally both for energy and for fat synthesis which allows for an accumulation of fat. The fact that rats will eat a ration high in fat when showing polyneuritic symptoms while the intake of a vitamin low-sucrose ration is reduced, indicates that dietary deficiencies influence food choice. Barelare, Holt and Richter ('38) have demonstrated the increased appetite for fat by rats on a thiamin deficient diet through free choice experiments.

SUMMARY

The evidence indicates that when the carbohydrate component of a thiamin-free ration is isocalorically replaced by natural fats or synthetic fatty acid esters the resultant diet will alleviate the polyneuritic syndrome of thiamin deficient rats.

Female rats fed a high fat ration (39.4% fat) grew at good growth rates and had normal estrous cycles.

The livers of rats fed a high fat diet were as low in cocarboxylase content as were those of polyneuritic animals. The brain tissue of rats fed thiamin-deficient high-fat diets retained a greater reserve of vitamin containing coenzyme.

No marked differences have been observed between olive oil, coconut oil, autoclaved peanut oil, autoclaved lard and autoclaved cottonseed oil as judged by the growth records of the rats.

Synthetic fatty acid esters, tricaproin and triacetin, were also effective in alleviating the polyneuritic syndrome of thiamin deficient rats.

LITERATURE CITED

ARNOLD, A., AND C. A. ELVEHJEM 1938 Studies on the vitamin B_1 requirements of growing rats. J. Nutrition, vol. 15, p. 429.

BARELARE, B., JR., L. E. HOLT, JR. AND C. P. RICHTER 1938 Influence of vitamin deficiencies on appetite for particular foodstuffs. Am. J. Physiol., vol. 123, p. 7.

EVANS, H. M., AND S. LEPKOVSKY 1928 Sparing action of fat on the antineuritic vitamin. Science, vol. 68, p. 298.

EVANS, H. M., S. LEPKOVSKY AND E. A. MURPHY 1934 The sparing action of fat on vitamin B. VI. The influence of the levels of protein and vitamin G. J. Biol. Chem., vol. 107, p. 429.

FUNK, C. 1914 Studien über Beriberi. XI. Die rolle der Vitamine beim Kohlenhydratstoffwechsel. Zeit. f. physiol. Chem., Bd. 89, S. 378.

GREGORY, E., AND J. C. DRUMMOND 1932 A study of fat metabolism with special reference to nutrition on diets devoid of fat. Z. Vitaminforsch., vol. 1, p. 257.

KEMMERER, A. R., AND H. STEENBOCK 1933 A study of the sparing action of fats on the vitamin B content of animal tissues. J. Biol. Chem., vol. 103, p. 353.

LIPTON, M. A., V. R. POTTER AND C. A. ELVEHJEM 1938 The relation of vitamin B_1 to cocarboxylase. Biochem. J., vol. 32, p. 474.

SALMON, W. D., AND J. G. GOODMAN 1937 Aleviation of vitamin B deficiency in the rat by certain natural fats and synthetic esters. J. Nutrition, vol. 13, p. 477.

STIRN, F. E., AND A. ARNOLD 1938 The relation of dietary fat to the vitamin B_1 requirement of growing rats. J. Biol. Chem., vol. 123, p. cxvii.

WHIPPLE, D. V., AND C. F. CHURCH 1936 The composition of growth induced by vitamin B(B_1). J. Biol. Chem., vol. 114, p. cvii.

———— 1937 The effect of vitamin B_1 on the respiratory quotient. J. Biol. Chem., vol. 119, p. ciii.

THE HEAT PRODUCTION AND BLOOD AND URINE CONSTITUENTS AFTER ADMINISTRATION OF d-LYSINE MONOHYDROCHLORIDE TO THE DOG

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Although the literature dealing with amino acids is quite large, there yet remain considerable gaps in our knowledge of the basic physiological properties of many of these compounds. To cite briefly only one phase of our incomplete information, we may point out the fact that five of the ten amino acids now generally conceded to be nutritional essentials (Rose, '38), isoleucine, methionine, tryptophane, lysine and threonine have never been individually tested to determine their effect upon heat production.

Inasmuch as we have had available adequate quantities of lysine, arginine and histidine, it seemed highly desirable to add to the existing data by comparatively detailed studies of the rates of metabolism of these acids and their effects upon the heat production of normal animals. Incidentally such experiments would seem to be a very valuable preliminary to maintenance of an animal by completely parenteral administration of amino nitrogen.

Although lysine has long been recognized as an essential amino acid, little is known concerning its intermediary metabolism. Dakin ('13) has shown that it produces neither extra glucose nor β-hydroxy butyric acid in the phlorhizinized dog. Rapport and Beard ('28) considered arginine responsible for most of the specific dynamic action of the basic amino acid

fraction of proteins, but owing to the high cost of lysine they were unable to make a direct test of this substance. So far as we are aware, no one has attempted such a study since that time.

METHODS

Dogs thoroughly trained for metabolic experiments were used in this investigation. They were maintained throughout on a constant diet and fasted at least 16 hours previous to each experiment. The experiments were all performed at room temperatures of 25 to 30°C., which is within the range of thermal neutrality for the dog.

Several determinations of basal metabolic rate and also of blood amino and urea nitrogen were obtained in a control period preceding the administration of the amino acid. Blood urea and amino nitrogen levels, as well as heat production, were determined hourly during a 6-hour experimental period following lysine administration. Heat production was measured by the Tissot-Haldane method, urea by the manometric urease method of Van Slyke ('27) and amino nitrogen by the manometric method of Van Slyke ('29). Urine collections were begun and terminated by catheterization. The control period was of approximately 2 hours duration, while the experimental period lasted about 6 hours. Both control and experimental samples were analyzed for total nitrogen by the Kjeldahl method, for urea by the manometric urease method of Van Slyke ('27) and for amino nitrogen by the manometric method of Van Slyke ('29). The amino nitrogen of the urine was determined directly after removal of urea and also, in some cases, upon the phosphotungstic acid precipitates.

In three experiments amytal anesthesia was used. However well trained a dog may be, there are times when slight movements will occur which will increase the heat production slightly but appreciably. This does not occur in dogs under deep amytal anesthesia and the specific dynamic action is unchanged (Eaton, Cordill and Gouaux, '36). Thus, even a small increase in heat production would represent a true specific dynamic action.

Four experiments were performed with glycine to serve as a check on the methods used and to provide a basis for comparison with the lysine studies. The amino acids for intravenous injection were dissolved in about 60 cc. of water, sterilized by heat and injected at a constant rate into a saphenous vein during a period of approximately 10 minutes.

RESULTS

Blood constituents. Average values for blood amino nitrogen following the administration of glycine and lysine are presented in table 1. It will be seen that the blood amino

TABLE 1

Average values for blood amino nitrogen and blood urea nitrogen after administration of glycine or lysine

NUMBER OF EXPERIMENTS	AMINO ACID GIVEN	METHOD OF ADMINISTRATION	AMINO NITROGEN GIVEN	MILLIGRAMS PER 100 CC. OF BLOOD						
				Control period (hours before injection)		Experimental period (hours after injection)				
				1	0.5	1	2	3	4	5
Amino nitrogen										
			gm.							
4	Glycine	Intravenous	1.796	9.8	9.5	29.4	13.9	11.2	11.6	10.8
2	Lysine	Intravenous	1.296	9.7	9.5	19.7	13.5	12.0	12.0	12.5
6	Lysine	Intraperitoneal	1.466	9.1	9.1	19.6	14.2	12.5	11.4	11.5
Urea nitrogen										
4	Glycine	Intravenous	1.796	11.6	12.0	15.7	19.3	19.9	17.6	15.9
2	Lysine	Intravenous	1.296	12.9	13.6	13.1	13.8	13.1	12.2	11.5
6	Lysine	Intraperitoneal	1.466	12.2	12.2	12.0	13.0	14.7	14.8	14.2

nitrogen level 1 hour after intravenous injection of glycine was very much higher than at the same time following lysine injection. This difference is due principally to the larger amount of nitrogen given in the glycine experiments. Even after allowing for the difference in the amount of nitrogen, however, we find that the lysine is eliminated from the blood somewhat more rapidly than the glycine during the first hour when the concentration is high, and somewhat less rapidly than the glycine during the third, fourth and fifth hours when the concentration is approaching the control figure.

From an examination of table 1 it is also evident that lysine is metabolized more slowly than glycine, since the blood urea nitrogen increase after lysine is very small in contrast to the large increase after giving glycine. These two results taken together indicate that, when a relatively large amount of lysine is injected directly into the blood stream, the compound is quite rapidly excreted and only a small fractional portion is utilized. This fact is strikingly apparent from the data in table 2. In the two intravenous experiments an aver-

TABLE 2

Average urea and amino nitrogen excretion after administration of glycine or lysine

NUMBER OF EXPERIMENTS	AMINO ACID	METHOD OF ADMINISTRATION	AMINO NITROGEN GIVEN	EXCESS URINE UREA NITROGEN	EXCESS URINE AMINO NITROGEN	
					Total	Diamino
			gm.	*gm.*	*gm.*	*gm.*
4	Glycine	Intravenous	1.790	0.714	0.563	
2	Lysine	Intravenous	1.296	0.524	1.020	
1	Glycine	Intraperitoneal	1.790	0.780	0.356	
6	Lysine	Intraperitoneal	1.466	0.347	0.629	0.437

age of only 276 mg. of the amino nitrogen (equivalent to 1.8 gm. of lysine monohydrochloride) were retained by the animal to undergo metabolism. For this reason we resorted to intraperitoneal injections in the hope that the compound would be absorbed more slowly and hence be retained in the body for a sufficient period of time to allow more nearly complete utilization. This hope was partially realized. The blood amino nitrogen value during the second hour is probably better maintained by the latter method. There is also a slight but significant increase in the blood urea nitrogen using this technic. Lysine, in this case, was being deaminized slightly faster than the kidneys eliminated the urea.

It is an interesting coincidence that in the dog lysine is metabolized slowly and is readily excreted when its concentration in the blood rises appreciably and that (Doty and Eaton, '37) the rate of absorption from the gastro-intestinal tract (as determined on rats) also proceeds at a slow rate as compared with other amino acids. If the same relative absorp-

tion rate is found for dogs, one might be tempted to infer the presence of a mechanism for supplying the organism with this amino acid at a rate at which it can be utilized efficiently.

Urinary constituents. The data summarized in table 2 are largely self-explanatory. The excess urine urea nitrogen after an intravenous administration of lysine was greater than after an intraperitoneal injection. This does not, however, indicate a more rapid rate of urea formation in the former case. Thus an average of 79% of the intravenously administered amino nitrogen appeared in the urine as such, while the extra urea nitrogen excreted, if derived from the lysine, would account for 40% of the amino acid nitrogen injected. In other words, the extra urinary urea nitrogen was two times as large as the amino nitrogen retained by the dog. It seems obvious that the marked diuretic action of the lysine, when it was placed directly in the blood stream, resulted in the washing out of considerable urea in excess of that arising from metabolism of lysine. This is further borne out by the blood urea nitrogen figures from table 1. It will be noted that the blood urea nitrogen actually fell (after intravenous injection of lysine) about 2 mg. per cent during the period of the experiment. Hence the urine values following the intraperitoneal injection must be regarded as more nearly representative of the urea formed in the metabolism of lysine.

When this amino acid was given by the latter method, an average of 43% of its nitrogen was excreted into the urine as amino nitrogen. Extra urea was equivalent to 24 of the remaining 57%. The amount of extra ammonia was in all cases too small to be of any significance. Furthermore, the extra total nitrogen as determined by the Kjeldahl method had a tendency to be slightly lower than the sum of the extra urea and amino nitrogen values. There was, then, at least 33% of the intraperitoneally administered nitrogen which could not be accounted for. That this discrepancy was due, in any considerable degree, to delayed absorption of the lysine from the peritoneal cavity does not seem probable in view of the findings regarding blood amino nitrogen. In table 1 it

can be seen that the amino nitrogen values of the blood rose rapidly during the first hour and by the end of the third or fourth hours had subsided to a rather constant level only slightly above the control level. We feel justified, therefore, in assuming that the greater part of the lysine reached the blood stream, that about one-fourth of its nitrogen was transformed to urea nitrogen, that there was at least a temporary storage of about one-third of its nitrogen and that the remainder of its nitrogen was excreted as amino nitrogen.

One other point deserves special mention. In the six intraperitoneal experiments the amino nitrogen of the urine was estimated both after removal of urea and following precipitation with phosphotungstic acid. There is considerable discrepancy between the two values. It would appear that nearly one-third of the excess amino nitrogen of the urine appeared in some form other than lysine. Further studies of this phenomenon will soon be instituted.

TABLE 3

Heat production after administration of glycine or lysine

DOG NUMBER	WEIGHT	CONTROL PERIOD		EXPERIMENTAL PERIOD			EXCESS URINE UREA N	AMINO ACID METABOLIZED	S.D.A.
		Average B.M.R.	Basal urine urea N	Amino acid used	Average metabolic rate	Urine Urea N			
Intravenous injection									
	Kilos	*Cal./hr.*	*mg./hr.*	*gm.*	*Cal./hr.*	*mg./hr.*	*mg.*	*m. Mols.*	*Cal./m.Mol.*
1	11.4	16.70	89.8	G 9.6	19.11	219.6	687	49.06	0.245
1	11.4	18.92	104.7	G 9.6	21.17	236.0	659	47.07	0.239
1	11.3	18.15	87.0	G 9.6	20.87	248.5	832	59.43	0.230
1	11.0	18.43	33.7	G 9.6	20.98	163.8	677	48.36	0.264
Average									0.244
1	10.8	17.88	78.1	L 8.45	18.40[1]	170.3	458	16.36	0.159[1]
Intraperitoneal injection									
1	11.1	17.81	47.5	L 8.86	17.75	99.0	243	8.68	—0.034
Intraperitoneal injection (amytal anesthesia)									
3	11.4	17.39	142.0	L 9.56	17.55	205.4	324	11.57	0.069
2	9.8	15.95	65.5	L 9.56	15.87	143.2	388	13.84	—0.029
3	10.3	15.81	40.9	L 9.56	15.71	126.2	438	15.65	—0.051
Average									—0.004

[1] Rise in second hour period only; dog restless.

Heat production. The specific dynamic action of glycine is in good agreement with the values previously reported (Eaton, Cordill and Gouaux, '36, and Wilhelmj and Mann, '30). In only two of the experiments on lysine was there any increase in heat production (table 3). In one of these the increase was so slight as to be insignificant. One other experiment showed a rise which was appreciable, however, only in the second hourly period when the dog displayed some restlessness. Thus this experiment cannot be regarded as indicating any dynamic action. We can only say that if lysine does possess a specific dynamic action, it is too low to be measured by the usual methods.

Although all attempts to administer lysine by stomach tube resulted in vomiting, we cannot say that lysine showed any toxic effects. Dog 1 received lysine on four occasions extending over a period of 19 days. That no serious kidney damage occurred is indicated by a rather constant and low blood urea nitrogen value throughout this period. The dog appeared in excellent health throughout. Our results do not support the findings of Newburgh and Marsh ('25) that lysine is definitely nephrotoxic.

SUMMARY

Lysine is metabolized much more slowly than glycine. It is readily excreted by the kidneys if present in the blood in large quantities.

So far as we are able to determine, lysine does not produce a specific dynamic action.

LITERATURE CITED

DAKIN, H. D. 1913 Studies on the intermediary metabolism of the amino acids. J. Biol. Chem., vol. 14, p. 321.

DOTY, J. R., AND A. G. EATON 1937 Metabolism of the basic amino acids. I. Rates of absorption in ratio of the monohydrochlorides of d-lysine and l-histidine. J. Biol. Chem., vol. 122, p. 139.

EATON, A. G., S. C. CORDILL AND J. L. GOUAUX 1936 The specific dynamic action of glycine intravenously administered to nephrectomized dogs. J. Nutrition, vol. 12, p. 113.

NEWBURGH, L. H., AND P. L. MARSH 1925 Renal injuries by amino acids. Arch. Int. Med., vol. 36, p. 682.

RAPPORT, D., AND H. H. BEARD 1928 The effects of protein split-products upon metabolism. III. Further investigation of the fractionated protein hydrolysates and of amino acids, and their relation to the specific dynamic action of the proteins. J. Biol. Chem., vol. 80, p. 413.

ROSE, W. C. 1938 The nutritive significance of the amino acids. Physiol. Rev., vol. 18, p. 109.

VAN SLYKE, D. D. 1927 Determination of urea by gasometric measurement of the carbon dioxide formed by the action of urease. J. Biol. Chem., vol. 73, p. 695.

———— 1929 Manometric determination of primary amino nitrogen and its application to blood analysis. J. Biol. Chem., vol. 83, p. 425.

WILHELMJ, C. M., AND F. C. MANN 1930 The influence of nutrition on the response to certain amino acids. I. The effect of fasting. Am. J. Physiol., vol. 93, p. 69.

THE HEAT PRODUCTION AND BLOOD AND URINE CONSTITUENTS AFTER ADMINISTRATION OF d-ARGININE MONOHYDROCHLORIDE TO THE DOG

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Although many papers dealing with arginine have appeared from time to time, there seem to be very few data in the literature concerning the effect of administered arginine on the heat production, the blood constituents and the nitrogenous constituents of the urine.

Thompson ('05 a and b) studied the nitrogenous constituents of the urine both after feeding and after injecting arginine. In most of the experiments the greater part of the arginine nitrogen could be recovered from the urine, chiefly in the form of urea. There was also some increase in the ammonia of the urine amounting to the equivalent of about 10% of the arginine nitrogen. Upon feeding arginine, none appeared in the feces, and in fact there was a decrease in fecal nitrogen on the experimental days. Arginine, ornithine and putrescine could not be detected in either urine or feces. The intravenous injection of arginine produced no toxic effects.

Rapport and Beard ('28) in a single experiment administered 5 gm. of arginine carbonate to a dog. They observed an increase in the heat production and in the urinary nitrogen. Kiech, Luck and Smith ('31) studied the rate of catabolism of arginine injected into rats. They found this substance to be catabolized rapidly. Ornithine, if formed, must have been utilized almost as rapidly, since at no time could more than

11% of the arginine nitrogen be unaccounted for as either arginine or urea.

The present study is an attempt to extend the earlier work quoted above and to add further data concerning the rate of utilization of arginine.

METHODS

With but few exceptions the plan of experiment and the methods of analysis have already been described (Doty and Eaton, '39). Urinary amino nitrogen was determined directly on the urine (after removal of urea) and also upon the silver arginine precipitate prepared according to Block ('34). Ammonia was determined by the method of Folin.

In two of the experiments reported here, 9.9 gm. of the arginine monohydrochloride were administered intravenously, and in two cases the same quantity was given by the oral route. In another experiment in which the amino acid was injected intravenously the changes in blood and urine nitrogen constituents were noted, but changes in heat production were not measured. For intravenous administration, the material was dissolved in about 60 cc. of water, sterilized by heat and injected into a saphenous vein at a constant rate during a period of 10 minutes. An attempt to administer a water solution of 20 gm. of the arginine by stomach tube was not successful since the dog lost at least a large part of the solution by vomiting. Our former experience had demonstrated that oral administration of amino acids in water solution often resulted in vomiting. Because of this fact, in experiment 18 we dissolved the arginine in a warm 2% solution of agar agar, allowed the agar to congeal and then forced the dog to swallow this solid material. A later test showed that the dog would retain 10 gm. of the amino acid in water solution when given by stomach tube. In experiment 19 this method of administration was used. The results of experiments 18 and 19 are entirely comparable, the only difference being that in the presence of the agar the compound is apparently absorbed at a somewhat slower rate.

RESULTS

Blood urea and amino nitrogen. The results of blood analyses are shown in table 1. The control values for both urea and amino nitrogen are well within normal limits for the dog. The amino nitrogen values in the two intravenous experiments show a marked similarity to the figures which Kiech, Luck and Smith ('31) obtained by analyzing the entire carcasses of rats after injection with arginine. Our amino nitrogen values tend to return to control levels in a shorter time, but

TABLE 1

Blood amino nitrogen and blood urea nitrogen values after administration of 9.9 gm. of arginine monohydrochloride

DOG NO.	EXPERIMENT NO.	METHOD OF ADMINISTRATION	MILLIGRAMS PER 100 CC. OF BLOOD						
			Control period (hours before administration)		Experimental period (hours after administration)				
			1	0.5	1	2	3	4	5
Amino nitrogen									
4	15	Intravenous	10.1	10.0	14.3	11.5	9.4	9.0	9.3
4	16	Intravenous	10.1	10.0	15.8	11.6	10.2	9.6	9.4
5	20	Intravenous	10.4	10.4	20.8	14.8	11.6	11.2	11.2
4	18	Oral (in agar)	8.1	8.0	8.0	8.4	9.0	9.4	9.4
4	19	Oral	8.3	8.3	9.1	10.2	10.5	9.6	8.7
Urea nitrogen									
4	15	Intravenous	13.6	13.7	17.8	20.5	22.6	21.0	18.0
4	16	Intravenous	13.3	13.3	16.8	19.8	22.8	22.2	21.3
5	20	Intravenous	6.1	6.3	11.6	15.1	17.4	17.1	16.1
4	18	Oral (in agar)	16.8	16.8	17.8	19.2	20.6	21.8	23.4
4	19	Oral	18.4	18.5	20.3	22.4	23.4	24.2	24.8

the dosage of amino acid per unit of body weight was somewhat lower. With oral administration changes in the curves are those to be expected when the rate of absorption is considered (Doty and Eaton, '38). After intravenous injections of arginine the blood urea values began rising promptly and attained their highest levels within 3 hours. Throughout the remainder of the experimental period the figures showed only a slight tendency to return to the control levels. The maxima are reached with both glycine and arginine at about the same

time but with glycine the decrease in blood urea is somewhat more rapid. The failure of blood urea nitrogen values to return to control levels soon after the termination of the specific dynamic action makes the interpretation of the heat production rise more difficult.

Urinary nitrogen. Unchanged arginine appeared in the urine in all experiments. Reference to table 2 shows that the quantity excreted after intravenous administration is fairly large and of about the same order as found in the case of glycine when it is given intravenously (Doty and Eaton, '39; Eaton, Cordill and Gouaux, '36). As would be expected, the

TABLE 2

Heat production and urinary nitrogen after administration of 9.9 gm. of arginine monohydrochloride

EXPERIMENT NO.	ARGININE EXCRETED	S.D.A. CAL.	EXCESS NON-ARGININE URINARY N	CAL./GM. OF EXCESS URINARY N	CAL./M M. METABOLIZED
	gm.		*gm.*		
15 (i.v.)	2.97	4.85	0.226	21.44	0.383
16 (i.v.)	2.88	3.35	0.144	23.26	0.415
18 (oral)	0.54	5.61	0.288	19.48	0.348
19 (oral)	0.63	6.88	0.441	15.60	0.279
Average				19.95	0.356

excretion following oral administration was very small. By this method the concentration of blood amino nitrogen never increased more than 2 mg. %. The figures for arginine excretion as given in table 2 were obtained by analyses of the silver precipitates. Figures so similar were obtained in each case from direct analyses of the urines after removal of urea that we may infer, as did Thompson ('05 b), that if ornithine is formed it is not excreted in appreciable quantities. It should be noted, in this connection, that both amino groups of ornithine are removed in the Van Slyke analysis and, hence, the amino nitrogen values after removal of urea would be much higher than those of the silver precipitates if any appreciable amount of ornithine were excreted. This was not the case. Kiech, Luck and Smith ('31) have inferred from their experiments on rats that ornithine, if formed, is probably almost as rapidly catabolized as is arginine itself.

The values given in table 2 for excess non-arginine urinary nitrogen are obtained by deducting four times the amino nitrogen from the Kjeldahl figures. Since they are in close agreement with the urinary urea nitrogen values the latter are not included in the table. In experiments 18 and 19 urinary ammonia nitrogen was determined. The excess was 74 and 86 mg. respectively, a much lower value than that reported by Thompson ('05 a).

Heat production. Our results prove conclusively that arginine produces a specific dynamic action. There are obvious objections to relating the increase in heat production to the excess urinary nitrogen or in comparing it with the stimulating action of glycine. One difficulty arises from the failure of the blood urea nitrogen to return to control level soon after termination of the specific dynamic action of the arginine. It has been demonstrated, by way of contrast, that an equal quantity of glycine is metabolized rapidly enough so that the blood urea values have returned to the control level in most cases by the end of a 5-hour experimental period (Doty and Eaton, '39; Eaton, Cordill and Gouaux, '36). Since with arginine all of the increase in heat production occurs within the first 4 or 5 hours, it would probably introduce an additional error to collect the urine for longer than the 6-hour period. We have calculated our results on the same basis as for glycine but call the attention of the reader to the fact that these values may be unavoidably higher than the true figures. On this basis arginine causes a much lower absolute rise in heat production than does an equal quantity of glycine. On the other hand, when expressed as Calories per millimol of arginine completely catabolized, the dynamic action of arginine is somewhat higher than that of glycine. One must, of course, make allowance on this basis for the high blood urea nitrogen at the end of the experimental period.

So far as we have been able to find, only a single experiment has been performed on the specific dynamic action of arginine. Rapport and Beard ('28) gave 5 gm. of arginine carbonate to a dog by mouth. They obtained an increase in

heat production of 5.7% above basal. If one recalculates their data on the basis used by us, one finds the value of 19.7 Cal./gm. of excess urinary nitrogen. This remarkably close agreement is most striking when one considers all of the unavoidable error which may creep into metabolic work and also when one realizes that different analytical methods were used.

We are not, as yet, prepared to discuss the specific dynamic action of arginine and its cause in relation to the possible participation of the guanidine group, ornithine or any other substance derived from the arginine molecule. We hope to have at our disposal a sufficient quantity of ornithine in the near future to extend our studies to this important substance.

SUMMARY

Upon either intravenous or oral administration of arginine monohydrochloride to the dog there is a prompt and long sustained rise in the urea nitrogen of the blood. There is an excretion of unchanged arginine in the urine reaching considerable proportions in the intravenous experiments. Arginine is metabolized somewhat more slowly than glycine.

Arginine exhibits a specific dynamic action, which calculated upon the basis of the amount administered, is less than that of glycine but calculated upon the basis of Calories per millimol deaminized is probably slightly higher than that of glycine.

Ornithine, if formed, does not appear in the urine in appreciable quantities.

No toxic effects of arginine were noted.

LITERATURE CITED

BLOCK, R. J. 1934 The determination of the basic amino acids in small quantities of protein by the silver precipitation method. J. Biol. Chem., vol. 106, p. 457.

DOTY, J. R., AND A. G. EATON 1938 Rate of absorption of the basic amino acids as a function of the amount fed. Am. J. Physiol., vol. 123, p. 53.

———— 1939 Metabolism of the basic amino acids. II. Studies on the heat production and blood and urine constituents after administration of d-lysine monohydrochloride to the dog. J. Nutrition, vol. 17, p. 1.

EATON, A. G., S. C. CORDILL AND J. L. GOUAUX 1936 The specific dynamic action of glycine intravenously administered to nephrectomized dogs. J. Nutrition, vol. 12, p. 113.

KIECH, V. C., J. M. LUCK AND A. E. SMITH 1931 Studies on arginine. I. The rate of catabolism of arginine in rats, including a method for the determination of arginine in biological material. J. Biol. Chem., vol. 90, p. 677.

RAPPORT, D., AND H. H. BEARD 1928 The effects of protein split-products upon metabolism. III. Further investigation of the fractionated protein hydrolysates and of amino acids, and their relation to the specific dynamic action of the proteins. J. Biol. Chem., vol. 80, p. 413.

THOMPSON, W. H. 1905 a The physiological effects of peptone and allied products. Part VI. The metabolism of arginin. J. Physiol., vol. 32, p. 137.

———— 1905 b The physiological effects of peptone and allied products. Part VII. The metabolism of arginin. J. Physiol., vol. 33, p. 106.